THE JOURNAL OF DAIRY RESEARCH

LONDON
Cambridge University Press
FETTER LANE E.C. 4

BOMBAY · CALCUTTA · MADRAS
Macmillan

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THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Dairy Research Committee of the Empire Marketing Board

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VOLUME IV

CAMBRIDGE
AT THE UNIVERSITY PRESS
1933

UNIVERSITY OF VIRGINIA LIBRARY

PRINTED IN GREAT BRITAIN

ERRATA

VOLUME IV, NUMBER 1

Page 48. *After*
J. Golding, J. Mackintosh and E. C. V. Mattick,
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The National Institute for Research in Dairying,
University of Reading.

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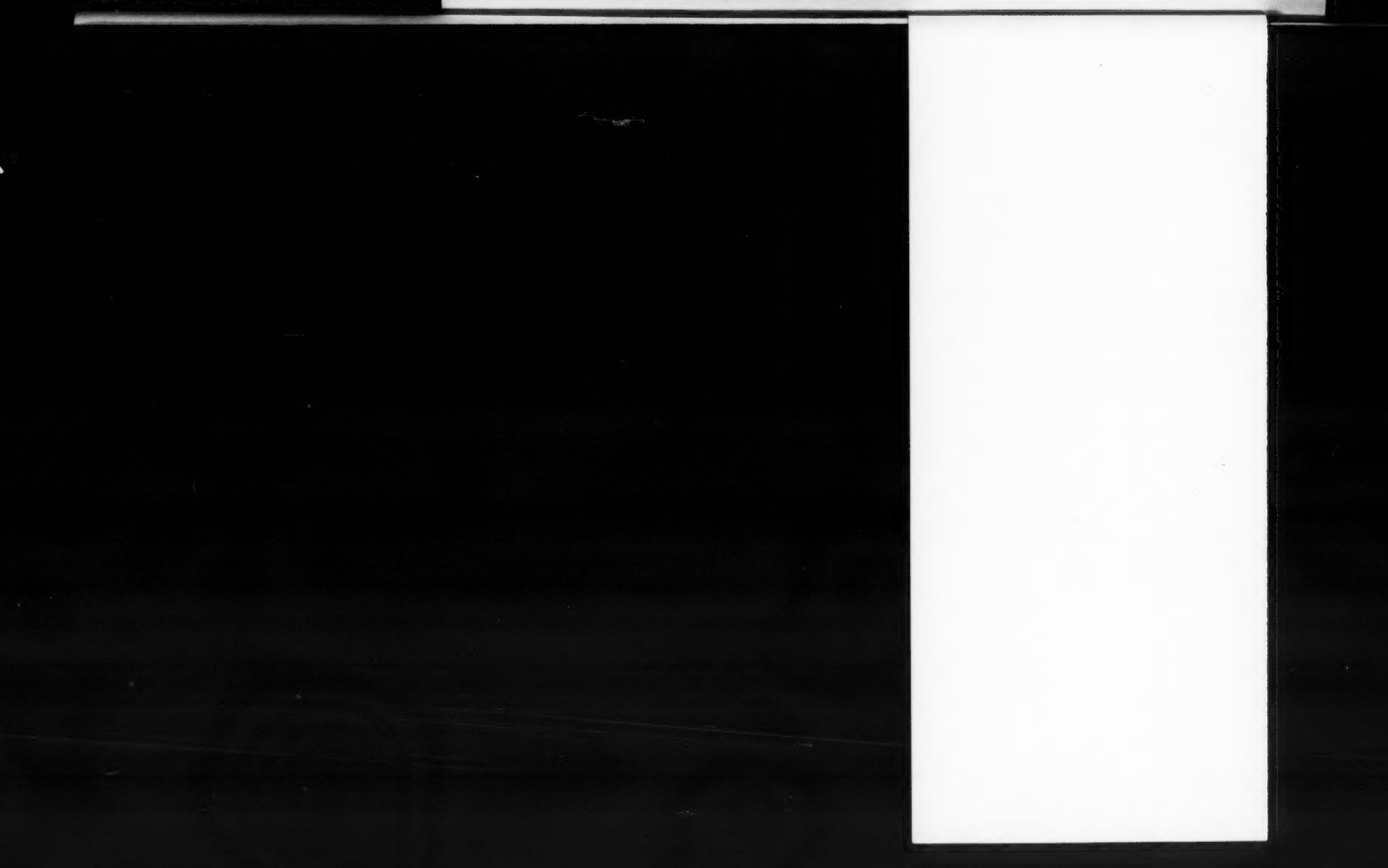

ABSTRACTS AND REVIEWS

Owing to the difficulty which has been experienced in providing sufficient space to include a comprehensive series of "Abstracts and Reviews," it has been decided to omit this section from the *Journal*. In its place the Editors have arranged to publish a series of Review articles, in which will be summarised the main advances which are made in the various branches of dairy science. For the present these Reviews, with comprehensive bibliographies, will be divided into five main sections, which will be published in the following issues:

Section A. The Physiology of Dairy Cattle (Vol. iv, No. 1).

Section B. Dairy Bacteriology (Vol. iv, No. 2).

Section C. Dairy Chemistry (Vol. v, No. 1).

Section D. The Diseases of Dairy Cattle (Vol. v, No. 2).

Section E. Milk-borne Infections, and the Nutritional Value of Milk (Vol. v, No. 2).

It is intended that each section shall subsequently be reviewed in rotation at two-yearly intervals.

The Editors would again remind subscribers that the *Monthly List of References from Current Literature*, issued by the National Institute for Research in Dairying, can be obtained free of charge on application to the Librarian, The National Institute for Research in Dairying, Shinfield, near Reading.

VOLUME IV DECEMBER, 1932 No. 1

637.121*

44. A STUDY OF SOME POINTS OF CONFORMATION AND MILK YIELD IN FRIESIAN COWS

BY FRANK H. GARNER,
School of Agriculture, Cambridge.

INTRODUCTION.

ALTHOUGH for centuries stock have been judged on appearances, the incidence of milk recording has revealed so many weaknesses in the method of judgment by the eye that it has become essential to ascertain which points are true indicators of potential milk-producing ability. Milk recording has made it possible to dispense, to some extent, with judgment of milking cattle by the eye, but inspection of external characters is still necessary when selecting heifer calves for rearing and bulls for breeding. In these cases the breeding and milk records of ancestors may be used, but the selection will ultimately depend on the external characters.

This study of the indicators of milk-producing ability in cows was begun with the object of finding those which are reliable, not only for cows, but also for heifers and bulls.

OUTLINE OF THE EXPERIMENTAL WORK.

A review of the literature showed that the subject had been studied in other countries, particularly in Germany, where a special staff for measuring cattle was used. This staff (Hauptner) consisted of a pole with two adjustable arms attached at right angles to it. A water bubble was fixed in the top of the staff to indicate whether it was being held vertically. It was graduated in centimetres and was suitable for measuring heights, lengths, depths and widths of animals, or parts of animals.

A survey of the literature revealed that in the past many different points of cows had been measured. The writer considered it impossible to measure some of these points accurately, and 27 points were selected. The preliminary trial consisted of measuring two Friesian cows (one fat and the other lean) 25 times each, to ascertain the accuracy with which these selected points could be measured. These measurements were taken on three consecutive days between 1 and 6 p.m. and recorded by an independent assistant. The standard deviations and coefficients of variations of the various parts measured were

* In Volume 4, Number 1, and all subsequent issues the Universal Decimal Classification will be adopted. The appropriate number under this Classification will appear at the right-hand top corner of the first page of each article. The numbers in this issue have been prepared with the help of the Science Library, South Kensington.

calculated for each cow, and these were compared with the coefficients of variation of 57 cows from six different herds.

Table I shows the coefficients of variation of each cow expressed as a ratio of those of 57 cows. The outstanding feature of the table is that the lean cow could be measured more accurately than the fat cow, for the fat made measurements more difficult. Only 19 of the original 27 selected points were measurable with sufficient accuracy to be studied, and for brevity these alone have been included in the table. The accuracy of measurement agrees very closely with the results of Lush and Copeland (11).

Table I. *Coefficients of variation of* 19 *body measurements of* (*a*) *fat cow,* (*b*) *lean cow, taken* 25 *times on each cow, and expressed as ratios of the coefficients of variation of* 57 *cows.*

Part measured	(*a*) Coefficients of variation of fat cow	(*a*) Coefficients of variation ratio, fat cow to 57 cows	(*b*) Coefficients of variation of lean cow	(*b*) Coefficients of variation ratio, lean cow to 57 cows
1. Length from withers to pins	1·156	3·235	0·517	7·236
2. Length from hooks to withers	1·709	2·660	0·928	4·897
3. Length from hooks to pins	0·927	4·083	0·764	4·952
4. Height at withers	0·434	7·143	0·244	12·708
5. Height between hooks	0·562	5·638	0·367	8·650
6. Height at hooks	0·721	4·307	0·493	6·303
7. Height at pins	0·604	5·914	0·312	11·426
8. Depth at chest	0·760	4·195	0·364	8·759
9. Width at chest	2·582	3·287	2·482	3·420
10. Width at barrel at 13th rib	2·116	3·742	2·015	3·931
11. Width at hooks	0·381	11·162	0·319	13·333
12. Width at thurls	0·702	6·718	0·864	5·463
13. Width at pins	2·929	2·476	0·929	7·808
14. Circumference of chest	0·266	13·105	0·251	13·938
15. Circumference of barrel	0·816	5·939	0·602	8·402
16. Barrel wedge	8·426	4·221	5·898	6·031
17. Total area of all milk wells	5·213	4·608	1·967	11·538
18. Double thickness of skin on ribs at 13th rib	2·902	3·778	1·995	5·495
19. Double thickness of skin on the udder	3·329	3·570	3·593	3·306

Nos. 1–13 inclusive were measured by means of the Hauptner staff. Nos. 14 and 15 were measured with a steel tape. No. 16 was obtained by subtracting No. 14 from No. 15. No. 17 was measured by a series of wooden plugs graduated according to area of cross section. Nos. 18 and 19 were measured by means of callipers.

The main experimental work was done at two centres with two different breeds of cow. In 1925–6 about 500 recorded Shorthorn cows in Berkshire were measured, but the figures had to be rejected because of the differences in the dairy and dual purpose types of this breed.

In 1926 and 1927 further measurements of pedigree and grade milk recorded Friesian cows of a uniform type found near St Paul, Minnesota, U.S.A., were made.

The writer's aim was to measure 500 mature cows, and for convenience "mature cows" was taken to include those over 4½ years old. When accurate

ages were unknown an estimate was obtained. All cows measured were tied up and measured in their stanchions. The cows were freshly moved just before any measurement was taken, for experience had shown that cows measure less in height after they have been standing for a time, than if they are measured immediately after a movement. For accuracy each part of each animal was measured twice, and the mean taken as the measurement; if any material discrepancy occurred, the measurement was taken a third time.

An effort was made at each farm to measure all mature milk recorded cows of the typical Friesian type which had not aborted and showed no malformations.

In addition to the measurements the following information was obtained for each cow:

Age of cow.
Dry period before last lactation.
Date of calving for last lactation.
Date served during last lactation.
Date dry after last lactation.
Milk produced during last lactation.
Butterfat produced during last lactation.

Also on each farm the system of management was studied by questions, in order to obtain an idea of the stimulus which the cows had received for milk production. In all some 20 questions were asked, but no satisfactory grouping according to management could be made.

RESULTS.

Before correlations could be calculated between the various body measurements and milk yields it was necessary to consider the question of correcting milk yields. The most suitable tables of figures for correcting lactation milk yields were those of Sanders (16) which were calculated from Shorthorn records and designed to correct yields for (*a*) age of the cow, (*b*) dry period before calving, (*c*) time of next service after calving, (*d*) time of year of calving. As the present data were for Friesian cows in Minnesota, U.S.A., and as Sanders' figures were for Shorthorns in parts of England it was considered necessary to test them on a few Friesians in Minnesota. If the correction figures were applicable, they should, if applied to several lactations of a particular cow, give a common yield for each lactation, provided the cow had remained healthy and had been fed uniformly throughout the whole period. The correction figures were applied to the yields of several old cows in the University of Minnesota herd with unsatisfactory results. Thus it was resolved to take the milk yields as produced by the cow, but to omit cows that had been in milk for more than 365 days, for it was considered that their milk yields would be abnormally high. This and other difficulties reduced the number of cows measured to 461.

The coefficients of correlation were calculated for the 19 different measurements and milk yield by the formula:

$$r = \frac{(XY)/N - \bar{X}\bar{Y}}{\sigma x\, \sigma y},$$

where x is one variable (milk yield), and y is the other variable (measurement), r = coefficient of correlation, $\bar{X}$ is the mean of X and $\bar{Y}$ the mean of Y.

From this the probable error was calculated by subtracting the correlation squared from 1 and dividing the result by $\sqrt{n}$, which is the factor showing the error for the limited population of cows used for the investigation:

$$Er = \frac{(1 - r^2)}{\sqrt{n}}.$$

Finally the correlation was expressed as a ratio to the probable error.

On account of the results obtained it was considered necessary to calculate a partial correlation between yield and the size of the milk well when a size factor was kept constant. This would show whether the milk well was large merely because the animal was big or whether it was because the milk flow was high. To make this study the coefficient of correlation between size and animal and size of milk well was obtained and then the following formula was used:

$${}_H r_{WM} = \frac{r_{WM} - r_{HW} r_{HM}}{\sqrt{1 - r_{HW}^2} \times \sqrt{1 - r_{HM}^2}},$$

where W = milk wells, M = milk yields, H = height.

The probable error of this coefficient of correlation was obtained by the formula:

$$E_{{}_H r_{WM}} = \frac{(1 - {}_H r_{WM}^2)}{\sqrt{n}}.$$

The results obtained are shown in Table II below.

Table II. *Coefficients of correlation between body measurements and milk production.*

Character	Coefficient of correlation	Probable error	Coefficient of correlation ÷ probable error
1. Length from withers to pins	+0·1946	±0·0302	6·44*
2. Length from hooks to withers	+0·1973	±0·0302	6·53*
3. Length from hooks to pins	+0·1031	±0·0311	3·31
4. Height at withers	+0·1313	±0·0313	4·19
5. Height between hooks	+0·1387	±0·0311	4·45
6. Height at hooks	+0·3881	±0·0269	14·43*
7. Height at pins	+0·3840	±0·0270	14·22*
8. Depth at chest	+0·1769	±0·0420	4·21
9. Width at chest	+0·0661	±0·0313	2·10
10. Width at barrel	+0·1711	±0·0305	5·60
11. Width at hooks	+0·1794	±0·0304	5·90
12. Width at thurls	+0·1422	±0·0308	4·61
13. Width at pins	+0·1050	±0·0311	3·37
14. Circumference of chest	+0·1923	±0·0303	6·35*
15. Circumference of barrel	+0·2716	±0·0292	9·30*
16. Barrel wedge	+0·1451	±0·0308	4·71
17. Size of milk wells	+0·3695	±0·0271	13·63*
18. Double thickness of skin on ribs	+0·0601	±0·0328	1·84
19. Double thickness of skin on the udder	+0·0257	±0·0331	0·78

There is some difference of opinion amongst statisticians regarding the degree of significance which is trustworthy. If a coefficient of correlation is at least six times its standard error it has been taken to be significant. In Table II seven measurements marked * have given significant results, while measurements Nos. 10 and 11 give coefficients of correlation 5·60 and 5·90 times their standard errors respectively, and may be considered as being, at least, suggestive. In this connection it should, however, be stated that it was difficult to take measurement No. 10 accurately because of the elasticity of the ribs and the enlargement of the barrel during pregnancy, so that error may inhibit a significant result. In addition a larger layer of fat may cover the hooks of some animals whereas with others the bones may be measured easily (see Table I).

In general the significant results indicate that the larger the Friesian cow the greater will her milk yield be. Sometimes size is most quickly measured by weighing an animal, but the difficulties of ascertaining the true live weights of cows have been discussed by Bartlett (2), Haecker (8), Beach (3), Jensen (9) and Wylie (20), who found that large cows gave more milk and butterfat than smaller cows. The most conclusive results were, however, obtained by Gowen (6) and Peters (14), each of whom studied nearly 2000 animals. Gowen gave the correlation of live weight with milk production of $+ 0{\cdot}652 \pm 0{\cdot}021$. Kronacher (10), with Baden cows, found the smaller cows were the better producers; but since this is a draught breed it is quite likely that the smaller animals would have a greater productive capacity. By actual measurements Woll (19), Dacy (4) and Rodenwald (15) found that the larger animals were the better producers.

If one turns to the significant individual measurements it will be noticed that they may be discussed as measurements indicating the following:

(1) Size of frame only.
(2) Size of frame indicating constitution.
(3) Size of frame indicating capacity.
(4) Measurements indicating mammary development.

(1) *Measurement of the size of frame.*

The significant measurements indicating size of frame are length of back, heights at hooks and pins, and widths at hooks. The importance of a cow with a long body is shown by the correlation coefficient of $+ 0{\cdot}1946 \pm 0{\cdot}0302$ (6·44 times its probable error), for it is in this length that all the parts vital for life, digestion and milk production are carried. This confirms the work of Mackenzie (12), with 80 Friesians, Jensen (9), Wylie (20) and Dolcini (5). Gowen (6) obtained a correlation coefficient between body length and milk yield of $+ 0{\cdot}590 \pm 0{\cdot}022$.

It is very difficult to understand from Table II why the heights of the hind-quarters of cows should be more highly correlated with milk yield than the heights of the fore-quarters. Gowen secured coefficients of correlation of

$+ 0{\cdot}380 \pm 0{\cdot}029$ for height at withers and $+ 0{\cdot}344 \pm 0{\cdot}030$ for height at hips. The only explanation which can be offered for the writer's figures is either a genetic one, *i.e.* parallel selection for shape and yield, or errors of measurement caused by differential settlement after movement. It is interesting to note that the coefficient of correlation between height at hooks and milk yield, *i.e.* $+ 0{\cdot}3881 \pm 0{\cdot}0269$, is much the same as that of Gowen. The results show the importance of length of hind leg as an indicator of potential milk production.

Effect of growth after the age of $4\frac{1}{2}$ *years.* It was considered that a little growth of frame might take place after $4\frac{1}{2}$ years, the age set as the lower limit for cows to be included. A special group of 150 cows between $7\frac{1}{2}$ and $11\frac{1}{2}$ years of age was therefore selected. This group gave a correlation coefficient, between milk yield and height at hooks, of $+ 0{\cdot}1646 \pm 0{\cdot}0794$ which is only 2·0730 times its probable error. The loss of significance is unquestionably due to increase in height after $4\frac{1}{2}$ years; further the probable error had increased, for approximately one-third of the number of cows in the larger study were in the smaller one.

Cattle judges prefer a cow with a large pelvis girdle, for they consider that the udder of a dairy cow must be suspended indirectly from four well-separated points, namely the hooks and the pins. It is interesting to notice that in Table II the coefficient of correlation between yield and width at hooks was $+ 0{\cdot}1794 \pm 0{\cdot}304$ (5·90 times its probable error). Gowen does not give figures for width at hooks, but he finds width at thurls and milk yield giving a correlation coefficient of $+ 0{\cdot}214 \pm 0{\cdot}033$.

(2) *Measurements indicating constitution.*

The chest measurement is generally taken to indicate that robustness of constitution which is necessary to meet the strain of milk production. Studies by Swett (18), give high correlations between external body measurements of chests and the weight of hearts and lungs, but it has not yet been shown that animals with relatively large lungs and hearts have particularly good constitutions. Constitution is, however, considered to be indicated by depth, width and circumference of chest. These points were measured and a significant correlation was obtained between circumference of chest and milk yield. The possible reason why this result was significant, whereas the depth and width of chest were insignificant, is that the circumference could be more accurately measured than the width or depth of chest. This was definitely so in the preliminary trial (Table I). On account of "ticklishness" depth of chest was measured on only 242 cows, and this is probably responsible for the loss of significance. The correlation coefficient of $+ 0{\cdot}1923 \pm 0{\cdot}0303$ obtained for chest circumference was not as high as Gowen's figure of $+ 0{\cdot}476 \pm 0{\cdot}027$.

(3) *Measurements indicating capacity.*

"Capacity" is really an abbreviation for capacity to consume and utilise large quantities of food. If a cow is to produce much milk it is assumed that she must consume a large amount of food, which in turn means that she must have a large barrel. In this work capacity was estimated by width of barrel at the thirteenth rib and by the circumference of the barrel. Although these measurements would be influenced by pregnancy and food and water consumption, a highly significant correlation coefficient was obtained for circumference of barrel and milk yield, namely $+ 0{\cdot}2716 \pm 0{\cdot}0292$, but only $+ 0{\cdot}1711 \pm 0{\cdot}0305$, or 5·60 times its probable error, for width at barrel and milk yield. Apparently few workers have studied barrel size and milk yield, but Wylie (20) with Jerseys found it to be the best single indicator of milking ability, whereas in this study it is the fourth best single indicator.

Another measurement which may be considered to indicate capacity is length of barrel (from withers to hooks) which gave a significant coefficient of correlation of $+ 0{\cdot}1973 \pm 0{\cdot}0302$, *i.e.* the coefficient was 6·53 times its probable error.

The above significant results stress the importance of adequate capacity in the better producing cows.

(4) *Measurements indicating mammary development.*

At this point it must be made perfectly clear that the total area of the milk wells of a cow was obtained by adding together all the milk wells on each side. Using this method, a high correlation between the size of milk wells and milk yield of $+ 0{\cdot}3695 \pm 0{\cdot}0271$ (13·63 times the probable error) was obtained. Aldrich and Dana (1) measured milk wells with their fingers and even with that crude method obtained correlation coefficients between diameter of milk wells and milk yield of $+ 0{\cdot}193 \pm 0{\cdot}036$ and $+ 0{\cdot}282 \pm 0{\cdot}027$. Jensen (9), by means of plugs, and Kronacher (10), Schmidt (17), McNatt and McKellip (13) and Dolcini (5) all found that higher producing cows had the larger milk wells.

It should also be pointed out, that, according to Graves (7), the blood leaves the udder by six different veins, but only two, the so-called milk veins, are visible. These veins, one on each side, run along the underside of the belly until they are within 6–12 in. of the fore-legs when they suddenly turn into the body cavity to the heart. Where the veins turn suddenly the milk wells are produced. It is assumed that the area of the milk veins is shown by the area of the milk wells, and that if they are large much blood must have been leaving the udder. Blood is required in the udder to provide the materials for milk production, and if much venous blood leaves the udder then much must have entered to have been used for milk production.

It was thought that the milk wells were larger on bigger cows, not because of higher milk yields, but to be proportionate to the frames of the cows. To

test this point it was considered necessary to calculate a partial correlation for which the formula shown on p. 4 was used and the results obtained are shown in Table III.

Table III. *Partial correlation of milk yield and size of milk wells with height at hooks held constant.*

Characters correlated	Coefficient of correlation	Probable error	Coefficient of correlation ÷ probable error
Height at hooks and milk yield	+0·3881	±0·0269	14·43
Size of milk wells and milk yield	+0·3695	±0·0271	13·63
Size of milk wells and height at hooks	+0·1178	±0·0313	3·76
Partial correlation: size of milk wells and milk yield with height at hooks held constant	+0·3538	±0·0278	12·72

It is interesting to observe in Table III that the size of the cow did not materially influence the size of the milk well, for the partial correlation between size of milk wells and milk yield, with height at hooks constant, was + 0·3538 ± 0·0278. The height at hooks was taken as a measurement of size which was correlated with milk yield. Thus the total area of the milk wells of a cow is an excellent indicator of her milk-producing ability, and with a little experience farmers may quickly find and measure milk wells with their fingers. Usually a large milk well will take the tip of the middle finger of a normal man's hand. Although one would never attempt to judge a cow by one point alone, the size of the milk wells is probably the best single point indicating milk-producing ability.

After the above study had been completed it was decided to calculate the correlation between size of milk wells and milk yield for cows between 7½ and 11½ years old, as was done for the height at hooks. This classification reduced the number of cows to 157, but the significant correlation coefficient was + 0·4040 ± 0·0662, or 6·10 times its probable error. This compares well with the correlation of + 0·3695 ± 0·0272 or 13·61 times its probable error for all cows. The coefficient of correlation is larger with the 157 cows, but the smaller number of cows increased the probable error. The figures show that for cows over 4½ years old milk wells are good indicators of milk-producing ability, since they do not increase in size because of mere growth of the cow but because of increased milk yield.

Since this was established the author has found that there is in fact a close correlation between size of milk wells and milk yields of cows and heifers of the British dairy breeds. Further, there may be a close relationship between the size of milk wells in bulls and the milking capacity of their female progeny.

Summary.

1. After a preliminary trial with Shorthorn cows in Berkshire, some 461 Friesians were measured in Minnesota, U.S.A., and an attempt was made to correlate 19 measurements with milk yields.

2. Significant coefficients of correlations were obtained for:
Length from withers to pins and milk yield;
Length from withers to hooks and milk yields;
Height at hooks and milk yield;
Height at pins and milk yield;
Circumference of chest and milk yield;
Circumference of barrel and milk yield;
Area of milk wells and milk yield.

3. Correlation coefficients that were almost significant were obtained for:
Width at barrel and milk yield;
Width at hooks and milk yield.

4. The best producing cows had the largest milk wells. This was not because they were the biggest cows, for there was practically no loss of coefficient of correlation for milk yield and size of milk wells when the partial correlation for area of milk wells and yield was calculated with height at hooks held constant.

5. Evidence is presented which shows that the areas of milk wells do not increase with mere skeletal growth of cows over 4½ years old, but with increased milk production. This fact makes the area of milk wells a still more reliable indicator of milk-producing power.

6. The results obtained confirm the value of some of the points which stockmen have considered in judging dairy cows. They emphasise the necessity for considering all the points together, but indicate that certain points deserve special consideration.

This work was carried out during the tenure of a Ministry of Agriculture scholarship.

The writer gratefully acknowledges the help he has received from the late Prof. T. B. Wood and from Mr James Mackintosh, Mr C. S. Orwin, Dr C. H. Eckles and the late Dr J. A. Harris and members of their respective staffs.

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(*MS. received for publication, February* 25, 1932.)

636.225.1:575.1

45. THE AYRSHIRE BREED OF CATTLE: A GENETIC STUDY

By ALEXANDER BURN FOWLER.
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(With 1 Figure).

Introduction.

Previous studies of the rôle of inbreeding in the development of pure breeds of cattle have been limited to those which have a long-standing history of pure breeding. Thus Wright (1) has made an analysis of inbreeding in Shorthorn cattle, which have been traced as a fairly pure breed as far back as 1800, while Buchanan Smith (2) has studied Jersey cattle, which have maintained a remarkable purity of breeding for close on 150 years. The present study is concerned with Ayrshire cattle, which constitute a breed of relatively recent establishment and of somewhat mixed origin.

The Ayrshire breed was undoubtedly derived from the indigenous cattle of the south-west of Scotland. These cattle were, however, graded up from time to time by the importations of cattle from England, notably from the Teeswater and Holstein breeds round about 1750. Another cross which left a great impression on Ayrshire cattle was the introduction of West Highland blood during the beginning of the nineteenth century. It was not until 1877 that the Ayrshire Cattle Herd Book Society was inaugurated, and authentic records of the breed became available for study.

It will be seen therefore that the Ayrshire cattle of the present day possess a somewhat mixed ancestry, and that existing types have a relatively brief history of pure breeding. Moreover the genetic construction of the breed is further complicated by a cleavage in the methods of individual breeders into two groups—those which breed for "vessel type" (with tight, flat vessels, and small corky perpendicularly hung teats), and those which concentrate on "milking type," irrespective of the minutiae of udder conformation. The Ayrshire breed constitutes, therefore, for the geneticist, an unusually interesting study, combining a relatively recent history of pure breeding with a present-day cleavage in breeding methods.

Objects.

The objects of the present study were as follows:

(1) *To study the construction of the Ayrshire breed, and the part contributed thereto by inbreeding.* Homozygosity is the result of inbreeding, and, as it is possible to determine the amount of inbreeding that has taken place in a

breed, a measure of the homozygosity can be obtained. Moreover, an investigation along these lines also provides an opportunity to study whether certain blood lines have played an important part in the construction of the breed, and to determine what special methods of breeding, if any, were practised by former breeders. It is also possible to compare the homozygosity of the breed with that of other breeds.

(2) *To determine whether any relation exists between inbreeding and productivity.* It has been the opinion of the majority of breeders that inbreeding has a detrimental effect on high milk production. This study has therefore been extended to include the amount of inbreeding of certain groups of high and average milk yielders of the Ayrshire breed, in order to compare these with the average for the breed as a whole.

Material.

The material for this study was procured from the following sources:

(1) The Ayrshire Herd Book Society's recorded pedigrees, the first volume of which was issued in 1878.

(2) The milk records were taken from the Annual Reports of the Scottish Milk Records Association for 1923 and 1928.

Methods.

The period over which authentic pedigrees were kept was taken as that period covered by the Ayrshire Herd Book Society's published volumes, namely from 1878 to 1927. Difficulties were met with in the endeavour to procure a representative sample of the populations. Methods used by workers in studying other breeds were found to be unsatisfactory when applied to the Ayrshire breed. Calder (3), in his study of the Clydesdale breed, took the first animal on the top of every fifth page of every fifth volume, while Wright's method (1) as applied to the Shorthorn breed took every animal whether male or female at the top of the page at regular intervals of so many pages and volumes. In the Ayrshire volumes, however, especially in the early editions, the number of animals entered on any one page differed as much as two to six, while later volumes had as many as eleven on one page.

It was decided therefore to take a set number of cows and bulls out of each fifth year volume, and this number was evenly distributed throughout the volume. The number taken was 75 cows and 75 bulls, a number which represents an average of nearly 6 per cent. of the total population in these volumes and is much in excess of any number previously worked out by other workers in this type of investigation.

The method employed in examining the pedigrees of the breed population was the "Approximate (or 'Short') Method of Calculating Coefficients of Inbreeding" (4) evolved by Sewall Wright and McPhee. In this method the sire and dam of the animal in question are recorded, and a random line of ancestry is obtained by letting the sequence of sires and dams which is to

be traced back in the herd book be heads and tails respectively in a coin-tossing experiment. The coefficient takes the values of 50 per cent. and 0 per cent., neglecting the effect of an inbred common ancestor. Wright also gives a method of estimating this effect. It was found, however, that in this study there were no inbred common ancestors, so that this part of the equation was dropped. As Wright pointed out, this method of determination means practically nothing so far as the individual is concerned, but by determining the proportion of ties in a sufficiently large random sample of a family or breed, a measure of the average degree of inbreeding of that family or breed can be obtained to as high a degree of accuracy as desired.

In the investigation of the pedigrees of high and average milk yielders, Wright's "Long Method"(5) was employed, the object being to give a better opportunity of studying the different blood lines, the systems of mating, and their relation to milk yield.

In this method the coefficient of inbreeding is obtained by a summation of the coefficients for every tie by which the parents are connected, each line tracing back from the sire to a common ancestor and thence forward to the dam, passing through no individual more than once. The same ancestor may of course be involved in more than one line. If the contribution of any one tie between the parents in a pedigree is F, and if fa is the coefficient of inbreeding of the common ancestor forming the tie, and n and n_1 the number of generations which separate the common ancestor from the sire and dam respectively, then

$$F = (\tfrac{1}{2})^{n+n_1+1}(1+fa).$$

RESULTS.

Coefficient of inbreeding for the whole breed.

Table I shows the amount of inbreeding in the Ayrshire breed over the period examined, these results also being shown in graphic form in Fig. 1.

The graph indicates the steady rise in inbreeding over the period 1877–1927. No inbreeding could be traced in the first two periods 1877–82, as the majority of the pedigrees examined consisted only of the sire and dam of the animals in question. These periods really cover the foundation of the pedigree stock, and dead animals were eligible for entry. In the third period, 1887, the pedigrees were still very incomplete and again indicate that no inbreeding was practised.

Inbreeding commenced in 1892 and has continued to increase up to the present day, when the average coefficient of inbreeding is probably about 5 per cent.

There was a distinct fall in 1917 with a corresponding rise in 1922 which may possibly be explained as due to the effect of the war period. In an endeavour to cut expenses a special register was kept for heifer calves during this period. These animals were only admitted into the later volumes after they produced a calf. This meant that later volumes not only contained their

own share of young animals eligible for entry but animals which would otherwise have been entered in previous volumes. These latter animals would be animals which were fully grown and which were thought worth while entering. It is quite possible, therefore, in view of the fact that inbreeding was on the increase, that this group may have been specially selected from a breeding standpoint.

Table I. *Inbreeding in Ayrshires by five-year intervals.*

Year	No. of animals	Average coefficient	Year	No. of animals	Average coefficient
1877	150	—	1907	150	2·00 ± 0·54
1882	150	—	1912	150	4·33 ± 0·77
1887	150	—	1917	150	3·66 ± 0·71
1892	150	2·66 ± 0·62*	1922	150	6·00 ± 0·89
1897	150	1·33 ± 0·44	1927	150	4·33 ± 0·77
1902	150	2·66 ± 0·62			

* Calculated as "probable error" throughout the paper.

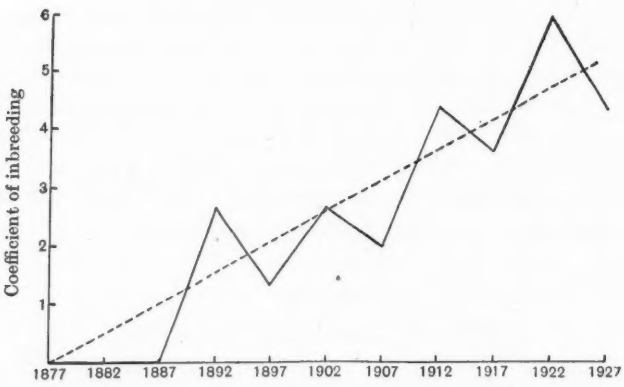


Fig. 1.

Effect of different foundation sires on the breed.

When working out the ties of the different periods the recurrence of certain animals from the Drumjoan herd was remarkable. It was found that, on summating the ties over the whole period 1877–1927, three bulls of the Drumjoan herd accounted for 25 per cent. of the inbreeding.

It was felt necessary at this stage to extend the pedigrees of the animals forming the ties so as to trace any possible relationship between them. This was done by Wright's "Long Method." Here again the results were very striking and showed the effect of some of the most prominent of the foundation sires.

The first bull to have an effective influence on the breed was Burnhouses (8), bred by Mr Lawrence Drew of Merryton. This bull was described by MacNeilage (6) as a "bull of great robustness of constitution, wide and roomy in

his shapes and, although not a show bull, full of style and breed character. His female progeny were full of style, with grand frames and rightly hung vessels, while their teats were rightly planted and of proper size. He was noted for his prize-winning stock of daughters who carried all before them, one of them being sold for £80 after winning the Edinburgh Highland Show in 1877."

It is obvious that an endeavour was made by certain breeders to breed for this type of animal through his grandson White Prince (63), although only one tie was traced directly to Burnhouses and one to another grandson, Loudoun Laddie (1069). White Prince was noted for the colour effect he had on the breed, leaving them mostly white. Three ties were traced to him directly and one to each of his two sons, Clansman (459) and Baron 3rd (458). Five ties were traced to Peter of Whitehill (1397), who was a great-grandson of White Prince on his sire's side. This bull was noted as the sire of good sound commercial animals with grand bodies, good vessels, and the right kind of serviceable commercial teats. He is said to have been a robust bull and never to have had an ailment during his long career. He was grandsire on the sire's side to Flora's Chief (3955), to whom three ties were traced.

The most famous foundation sire seems to be Hover-a-Blink (892), as he appears eight times as inbreeding sire. He was bred by Mr T. Hutchison, McCoshton. His sire, Outmains of Craigdhu (789), had only one tie traceable to him. Hover-a-Blink was a very prepotent breeder, and stock off him had great family likeness. In this study he has three sons with ties, the foremost being Cock-a-Bendie (1204) with six, the others, Cedric the Saxon (1306) and Blue Blood of Overton (v. 19), have one tie respectively.

He appears also on the sire's side of six other bulls to which inbreeding has been traced.

Diagrams 1 and 2 show the various connecting links between animals to which the Ayrshire breed is inbred and trace them back to the two foundation sires, namely Burnhouses (8) and Hover-a-Blink (892).

18·07 per cent. of the total inbreeding coefficient is traceable to Burnhouses or his offspring and 30·12 per cent. to Hover-a-Blink or his descendants.

Comparison of coefficient of bulls and cows.

Table II (*a*) shows the coefficient of inbreeding of bulls and cows in each fifth yearly period.

The significant difference in the inbreeding of cows as against bulls in 1892 may be the result of breeders in those early times trying to establish type by grading up their cows and inbreeding to certain sires.

By 1897 the inbreeding of both cows and bulls was equal, but by 1902 the cows registered were again more inbred and continued to be so until 1917, when, as already mentioned in discussing the breed average, only a limited number of heifers were entered during the war period, which resulted in an increased number of special animals being entered in 1922.

Diagram 1. *Blood lines traced to foundation sire.*

(Ties to the different animals are enclosed in brackets.)

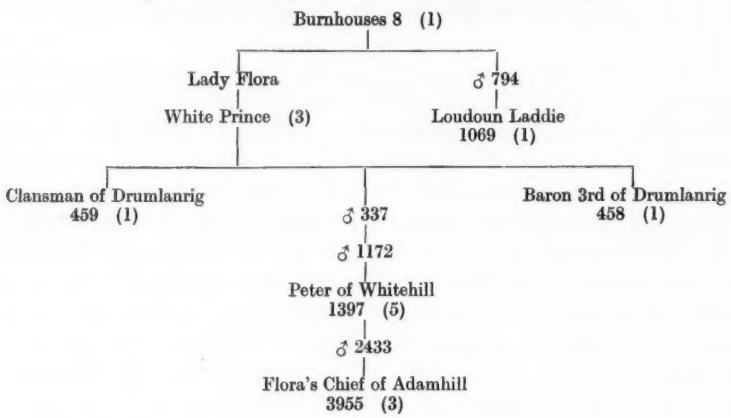
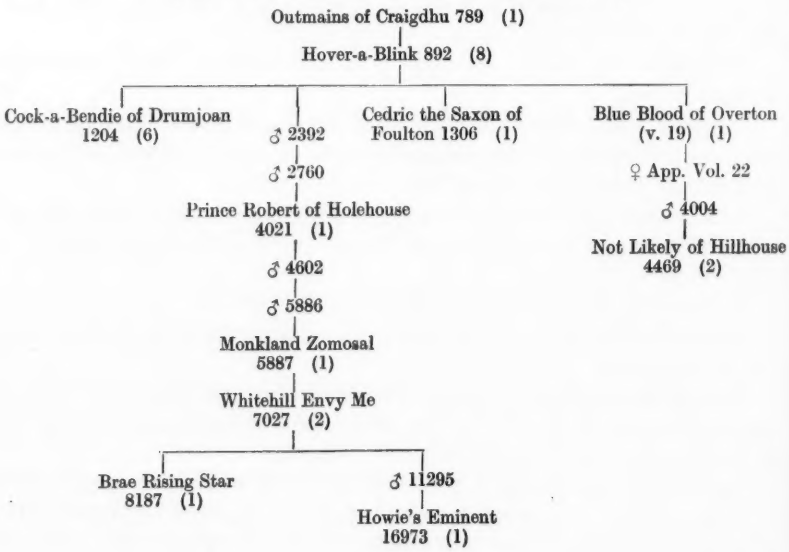


Diagram 2. *Blood lines traced to foundation sire.*

(Ties to the different animals are enclosed in brackets.)


Outmains of Craigdhu 789 (1)
Hover-a-Blink 892 (8)
Cock-a-Bendie of Drumjoan 1204 (6)
♂ 2392
Cedric the Saxon of Foulton 1306 (1)
Blue Blood of Overton (v. 19) (1)
♂ 2760
♀ App. Vol. 22
Prince Robert of Holehouse 4021 (1)
♂ 4004
Not Likely of Hillhouse 4469 (2)
♂ 4602
♂ 5886
Monkland Zomosal 5887 (1)
Whitehill Envy Me 7027 (2)
Brae Rising Star 8187 (1)
♂ 11295
Howie's Eminent 16973 (1)

With regard to the sharp rise in the inbreeding of bulls in 1922, it is significant that the animals to which the inbreeding was traced were related to the two great foundation sires, Burnhouses and Hover-a-Blink, as also were the animals to which the inbred cows were traced. During this period, when the outcry was raised against the "vessel-type" animal, and special shows were held to promote milk production, it is possible that breeders inbred to animals which were proved prepotent breeders of the milk type.

Owing to the difficulty experienced in finding a method of sampling the population, it was thought advisable to check the method of random sampling as a possible explanation of the differences of inbreeding between the bulls and the cows.

Table II. *Average coefficients of inbreeding of bulls and cows*, 1892–1927.

	Bulls		Cows	
Year	No. of animals	Average coefficient	No. of animals	Average coefficient
	(*a*) Original sample. Average coefficient of inbreeding.			
1877	75	—	75	—
1882	75	—	75	—
1887	75	—	75	—
1892	75	1·33±0·63	75	4·00±1·06
1897	75	1·33±0·63	75	1·33±0·63
1902	75	2·00±0·81	75	3·33±0·97
1907	75	2·00±0·81	75	4·00±1·06
1912	75	4·00±1·06	75	4·66±1·13
1917	75	4·66±1·13	75	2·66±0·87
1922	75	6·66±1·32	75	5·33±1·14
1927	75	4·00±1·06	75	4·66±1·13
	(*b*) Check samples. Average coefficient of inbreeding.			
1897	—	—	75	2·00±0·81
1907	75	0·66±0·45	—	—
1917	—	—	75	3·33±0·97
1922	75	2·00±0·81	—	—

Table II (*b*) is the result of the check samples, which correspond to 16 per cent. and 5 per cent. of the population in the case of the bulls, and to 14 per cent. and 18 per cent. in the case of the cows. The difference between the original samples and the check samples is somewhat difficult to explain. The results of the check samples are, however, not so surprising when it is taken into account that one-third of the animals entered in the herd books are traced to dams which are only once or twice removed from non-pedigree stock on the dam's side. It would appear that in pedigree work of this kind, where the traceable pedigrees are so short, the "short method" of calculating the coefficient of inbreeding leaves too much to chance. There is, therefore, a disadvantage in employing the "short method" as against the "long method" in a breed analysis where the breed is of such recent origin as the Ayrshire breed. The "long method" would give better results, as it would be possible to compare the extended pedigree of the dam's sire with the extended pedigrees of the sire's sire and dam, even though the dam's dam

were not traceable. This was actually found to be the case when the pedigrees of the animals to which ties were traced were examined by the "long method." Inbreeding to Burnhouses and Hover-a-Blink was found to be quite intensive, whereas the "short method" failed to reveal any inbreeding to these animals.

Productivity.

In order to discover any connection between milk yield and inbreeding, the pedigrees of three groups of milk yielders were examined.

The data were secured from the Scottish Milk Records *Reports* of 1923 and 1928. The object in selecting these years was, *inter alia*, to ascertain, if possible, the reason for the marked difference of inbreeding of the breed average in 1917 and 1922. The animals taken in the Milk Record *Reports* of 1923 and 1928 correspond to the herd book entries of 1917 and 1922 respectively.

To secure a representative sample of the high milking population, the first animal of 1000 gallons or over on every page was taken from the 1923 volume and its pedigree extended to the sixth generation. To find a group for comparison, 166 cows under 800 gallons were taken by choosing the first animal under 800 gallons on every third page of the same volume. The 800 gallon group could not be classed as low milk yielders, owing to the fact that the milk records of animals under 2800 gallons as cows and under 2240 gallons as heifers (all at 1 per cent. fat) are not published in the *Reports*. It was therefore decided to call this group average milk yielders. For check purposes, a further group of 102 animals of 1000 gallons or over was taken from the 1928 volume.

Table III.

	Short method			Long method	
Year of herd book entry	Breed average coefficient	Cow coefficient	Year of record	High milk yielders 1000 and over	Average milk yielders 800 and under
1917	3·66±0·71	2·66±0·87	1923	0·4579	0·5042
1922	6·00±0·89	5·33±1·14	1928	1·1466	—

Table III shows the percentage coefficient of the breed average and the three "milk recorded" groups. It will be seen that there is only a very slight difference between the coefficient of inbreeding for the high and average milk yielders of 1923, but that the coefficient for the high milk yielders of 1928 is appreciably higher than that of 1923.

In making a comparison with the breed as a whole it will be noticed that all three "milk recorded" groups are much less inbred than the breed average, but it will also be seen that the lower coefficient of 1923 "milk recorded" groups, as compared with the 1928 group, corresponds to the lower breed average of 1917 (and especially to the average coefficient of the cows of 1917) as compared with the breed average for 1922.

Table IV. *Showing the influence of certain herds on milk-producing groups.*

	1923. Group 1 166 animals 1000 galls. and over				1923. Group 2 166 animals 800 galls. and under				1928. Group 3 102 animals 1000 galls. and over			
	Total ties	% ties	Total coeffi-cient	% coeffi-cient	Total ties	% ties	Total coeffi-cient	% coeffi-cient	Total ties	% ties	Total coeffi-cient	% coeffi cient
Drumjoan	91	—	—	—	45	—	—	—	23	—	—	—
Wyndholm	6	—	—	—	2	—	—	—	5	—	—	—
Morton Mains	1	—	—	—	2	—	—	—	1	—	—	—
Drumjoan	98	39·04	13·95	18·36	49	21·12	9·022	10·78	29	14·26	15·14	12·95
Hillhouse	16	6·37	6·34	8·35	11	4·47	2·78	3·32	20	9·82	5·49	4·69
Whitehill	14	5·58	4·68	6·16	13	5·60	1·218	1·46	10	4·90	6·35	5·43
Auchenbrain	12	4·78	2·67	3·52	28	12·65	8·04	9·60	12	5·88	3·99	3·42
Drumlanrig	11	4·38	0·97	1·28	6	2·59	0·633	0·76	1	0·49	0·0487	0·04
Holehouse	9	3·58	2·54	3·33	7	3·02	1·41	1·69	8	3·92	1·60	1·38
Netherhall	8	3·19	1·90	2·50	9	3·88	1·66	1·98	8	3·92	0·93	0·78
Orchardton	6	2·39	4·00	5·27	5	2·16	0·34	0·41	6	2·94	0·88	0·75
Carston	6	2·39	1·22	1·60	—	—	—	—	—	—	—	—
Bogwood	6	2·39	1·22	1·60	3	1·29	0·5362	0·64	4	1·96	0·5362	0·46
Southwick	3	1·19	0·34	0·45	11	4·74	1·315	1·57	—	—	—	—
Carsegowan	3	1·19	0·34	0·45	9	3·88	1·61	1·92	5	2·45	0·39	0·33
Lessnessock	2	0·79	0·88	1·15	8	3·45	6·00	7·18	14	6·86	2·63	2·25
Bargenoch	2	0·79	0·82	1·09	3	1·29	10·94	13·07	18	8·82	5·95	5·09

Table IV shows the remarkable degree of inbreeding to the bulls of the different herds, the bulls of the Drumjoan herd again being foremost in this respect. The number of ties as well as the inbreeding coefficient is set down as it gives a better value of the extent to which the various bulls were used and their influence on the group. The inbreeding coefficient in itself gives rather a false picture of what took place in the group as, for instance, in 1923 the bull Craigs of Kyle (1793) has 39 ties but is only responsible for an inbreeding coefficient of 3·8512, whereas Combine (7305) has only 1 tie and is responsible for a coefficient of 6·25.

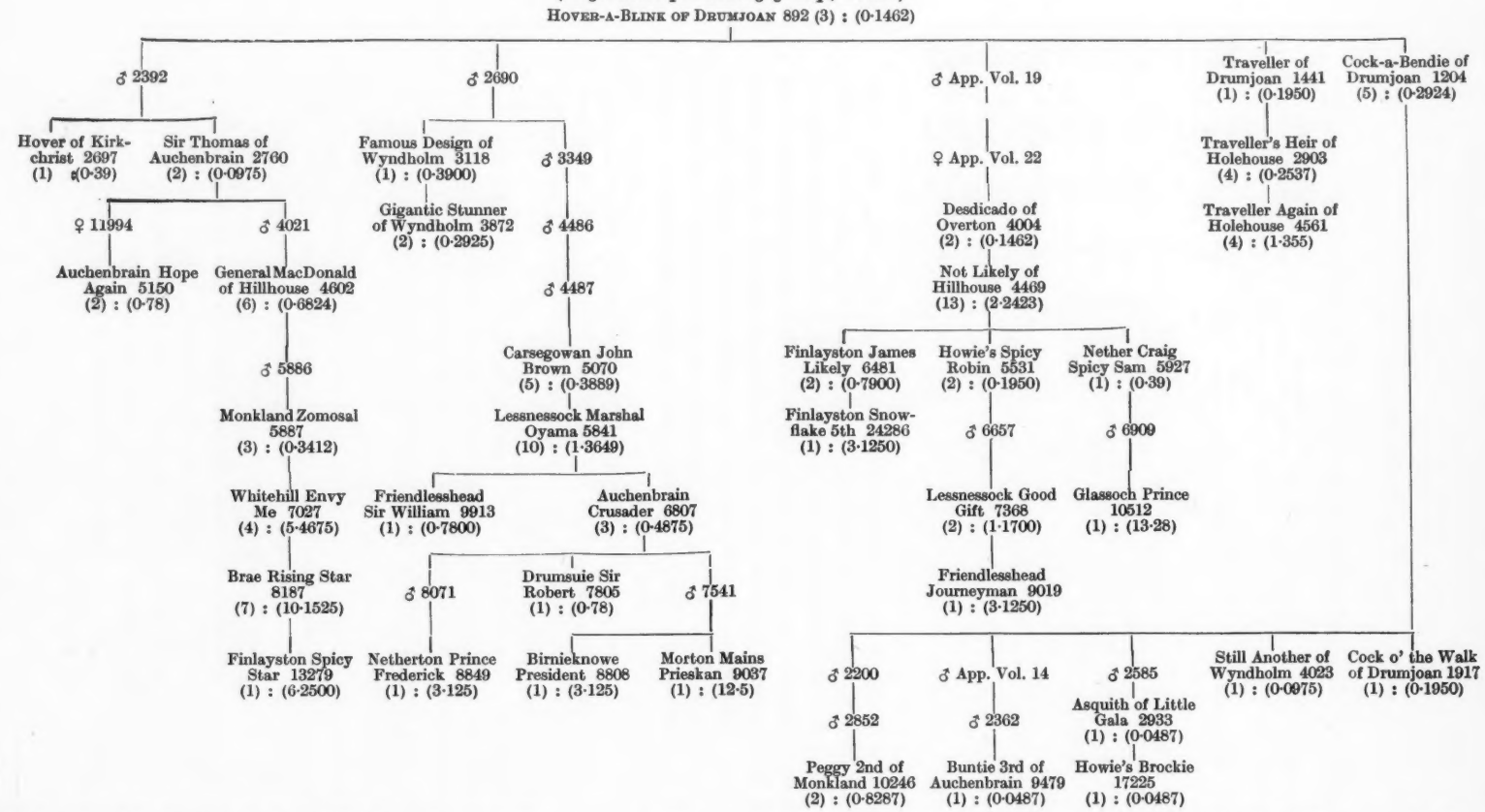
This herd first entered animals in the Herd Book in 1883 under the prefix Drumjoan, but was transferred to Wyndholm in 1893 and again transferred to Morton Mains in 1904. In the above table they were grouped together under the original prefix of Drumjoan.

Diagrams 3, 4 and 5 show the tremendous influence of the bull Hover-a-Blink on the milk-producing groups. The pedigrees of animals to which inbreeding has been traced were extended and all animals related to Hover-a-Blink were listed and their ties and coefficients summated. The results are shown in Table V.

Table V.

Year	Animals of 1000 gallons or over	Total number of ties for the group	Percentage of ties traced to animals related to Hover-a-Blink	Total coefficient for group	Percentage of total inbreeding coefficients traced to animals related to Hover-a-Blink
1928	102	204	49	101·16	64·36
1923	166	251	39·5	101·136	26·07
	Animals of 800 gallons or under				
1923	166	232	45·19	88·69	38·35

Diagram 3*. *Animals to which inbreeding was traced and which are related to Hover-a-Blink through sire's pedigree.*
(*High milk-producing group*, 1928.)


HOVER-A-BLINK OF DRUMJOAN 892 (3) : (0·1462)
♂ 2392
♂ 2690
♂ App. Vol. 19
Traveller of Drumjoan 1441 (1) : (0·1950)
Cock-a-Bendie of Drumjoan 1204 (5) : (0·2924)
Hover of Kirkchrist 2697 (1) : (0·39)
Sir Thomas of Auchenbrain 2760 (2) : (0·0975)
Famous Design of Wyndholm 3118 (1) : (0·3900)
♂ 3349
♀ App. Vol. 22
Traveller's Heir of Holehouse 2903 (4) : (0·2537)
♀ 11994
♂ 4021
Gigantic Stunner of Wyndholm 3872 (2) : (0·2925)
♂ 4486
Desdicado of Overton 4004 (2) : (0·1462)
Traveller Again of Holehouse 4561 (4) : (1·355)
Auchenbrain Hope Again 5150 (2) : (0·78)
General MacDonald of Hillhouse 4602 (6) : (0·6824)
♂ 4487
Not Likely of Hillhouse 4469 (13) : (2·2423)
♂ 5886
Carsegowan John Brown 5070 (5) : (0·3889)
Finlayston James Likely 6481 (2) : (0·7900)
Howie's Spicy Robin 5531 (2) : (0·1950)
Nether Craig Spicy Sam 5927 (1) : (0·39)
Monkland Zomosal 5887 (3) : (0·3412)
Lessnessock Marshal Oyama 5841 (10) : (1·3649)
Finlayston Snowflake 5th 24286 (1) : (3·1250)
♂ 6657
♂ 6909
Whitehill Envy Me 7027 (4) : (5·4675)
Friendlesshead Sir William 9913 (1) : (0·7800)
Auchenbrain Crusader 6807 (3) : (0·4875)
Lessnessock Good Gift 7368 (2) : (1·1700)
Glassoch Prince 10512 (1) : (13·28)
Brae Rising Star 8187 (7) : (10·1525)
♂ 8071
Drumsuie Sir Robert 7805 (1) : (0·78)
♂ 7541
Friendlesshead Journeyman 9019 (1) : (3·1250)
Finlayston Spicy Star 13279 (1) : (6·2500)
Netherton Prince Frederick 8849 (1) : (3·125)
Birnieknowe President 8808 (1) : (3·125)
Morton Mains Prieskan 9037 (1) : (12·5)
♂ 2200
♂ App. Vol. 14
♂ 2585
Still Another of Wyndholm 4023 (1) : (0·0975)
Cock o' the Walk of Drumjoan 1917 (1) : (0·1950)
♂ 2852
♂ 2362
Asquith of Little Gala 2933 (1) : (0·0487)
Peggy 2nd of Monkland 10246 (2) : (0·8287)
Buntie 3rd of Auchenbrain 9479 (1) : (0·0487)
Howie's Brockie 17225 (1) : (0·0487)

Total number of ties for group of animals 1000 gallons or over for 1928	...	...	204
Total percentage of ties traced to animals related to Hover-a-Blink	...	...	49·5

Total inbreeding coefficient for group of animals 1000 gallons or over for 1928	...	101·16
Total percentage inbreeding coefficient of animals related to Hover-a-Blink	...	64·36

* In Diagrams 3, 4 and 5, the first bracketed figure indicates the number of ties traced directly to the animal; the second bracketed figure is the total coefficient of inbreeding traced to the animal.

Diagram 4. *Animals to which inbreeding was traced and which are related to Hover-a-Blink through sire's pedigree.*

Total number of ties for group of animals 1000 gallons or over for 1923 251
Total percentage of ties traced to animals related to Hover-a-Blink ... ... 49·5

Total inbreeding coefficient for group of animals 1000 gallons or over for 1923 ... 101·136
Total percentage inbreeding coefficient of animals related to Hover-a-Blink ... ... 26·07

* In Diagrams 3, 4 and 5, the first bracketed figure indicates the number of ties traced directly to the animal; the second bracketed figure is the total coefficient of inbreeding traced to the animal.

Diagram 4. ***Animals to which inbreeding was traced and which are related to Hover-a-Blink through sire's pedigree.***
(High milk-producing group, 1923.)

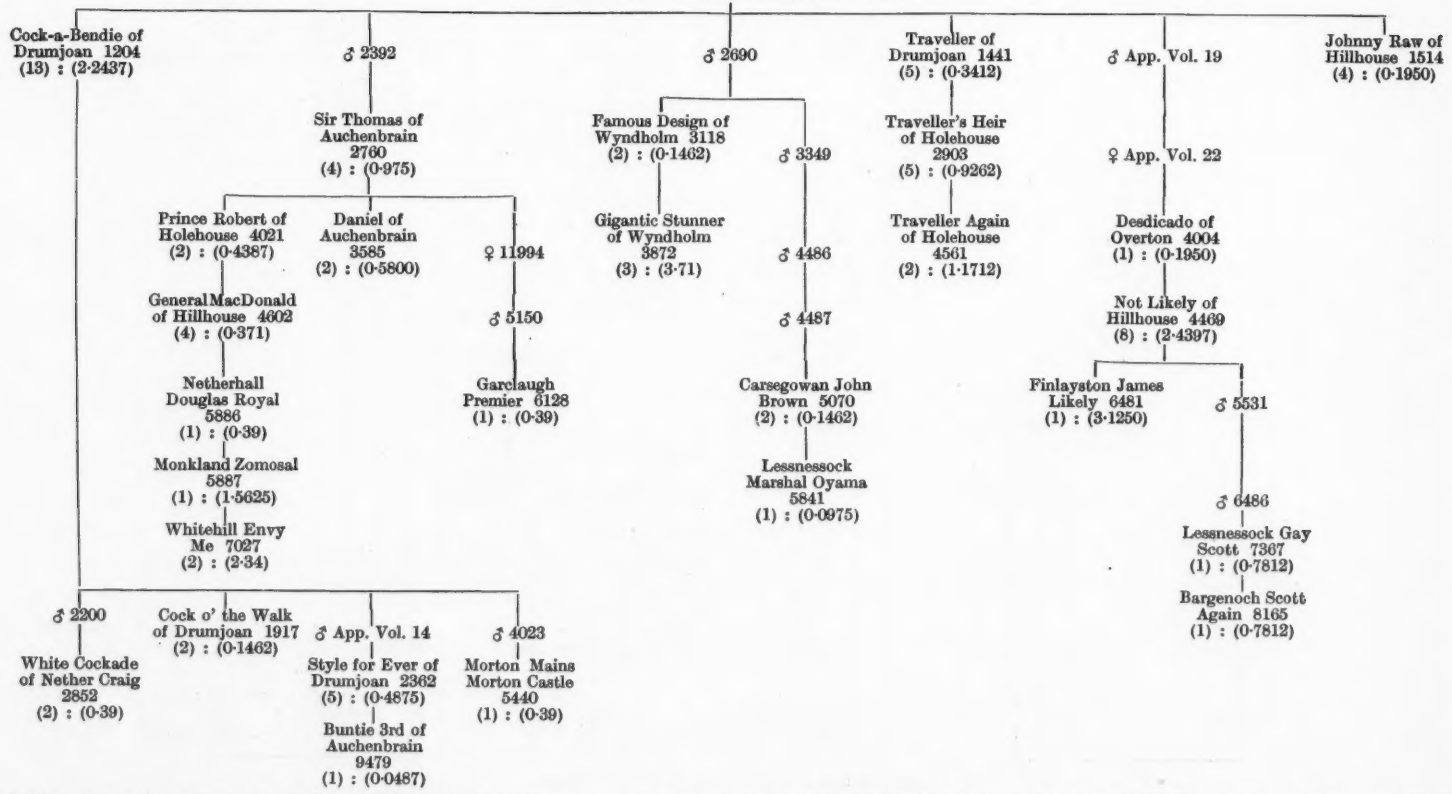
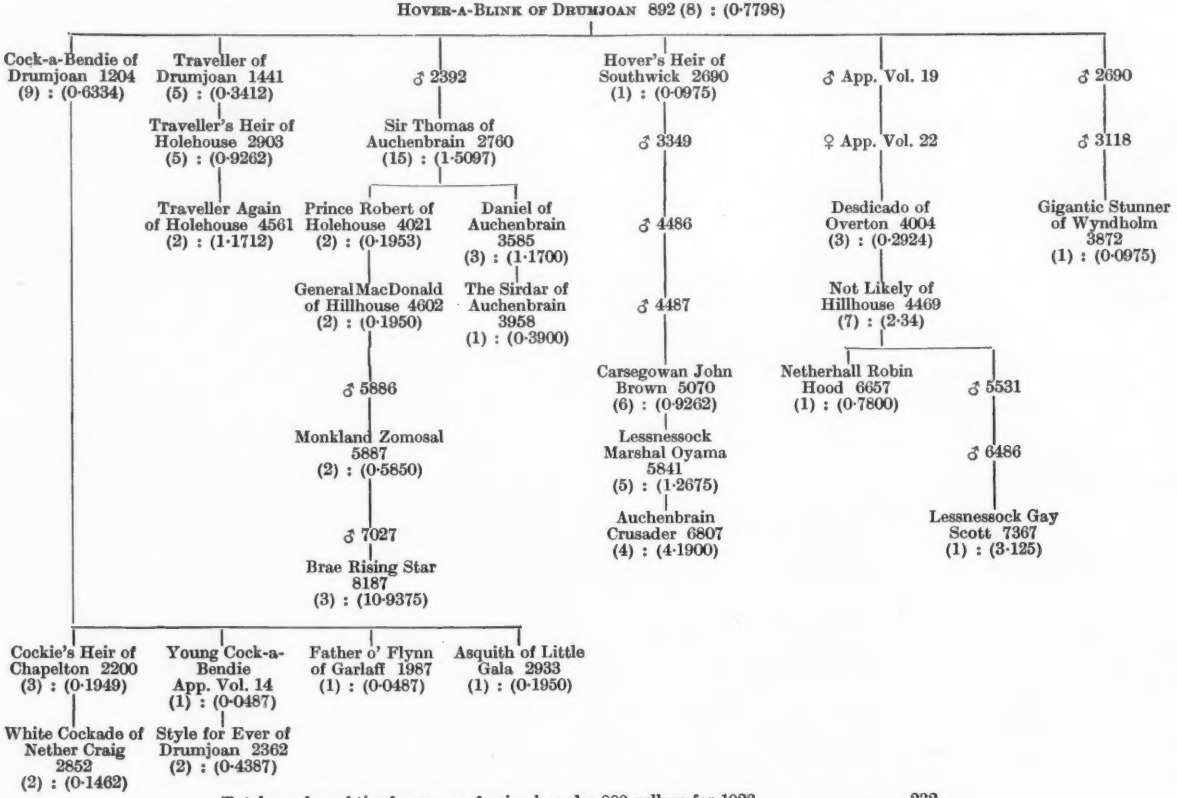

Hover-a-Blink of Drumjoan 892 (22) : (1·56)
Cock-a-Bendie of Drumjoan 1204 (13) : (2·2437)
♂ 2392
Sir Thomas of Auchenbrain 2760 (4) : (0·975)
Prince Robert of Holehouse 4021 (2) : (0·4387)
General MacDonald of Hillhouse 4602 (4) : (0·371)
Netherhall Douglas Royal 5886 (1) : (0·39)
Monkland Zomosal 5887 (1) : (1·5625)
Whitehill Envy Me 7027 (2) : (2·34)
Daniel of Auchenbrain 3585 (2) : (0·5800)
♀ 11994
♂ 5150
Garclaugh Premier 6128 (1) : (0·39)
♂ 2690
Famous Design of Wyndholm 3118 (2) : (0·1462)
Gigantic Stunner of Wyndholm 3872 (3) : (3·71)
♂ 3349
♂ 4486
♂ 4487
Carsegowan John Brown 5070 (2) : (0·1462)
Lessnessock Marshal Oyama 5841 (1) : (0·0975)
Traveller of Drumjoan 1441 (5) : (0·3412)
Traveller's Heir of Holehouse 2903 (5) : (0·9262)
Traveller Again of Holehouse 4561 (2) : (1·1712)
♂ App. Vol. 19
♀ App. Vol. 22
Desdicado of Overton 4004 (1) : (0·1950)
Not Likely of Hillhouse 4469 (8) : (2·4397)
Finlayston James Likely 6481 (1) : (3·1250)
♂ 5531
♂ 6486
Lessnessock Gay Scott 7367 (1) : (0·7812)
Bargenoch Scott Again 8165 (1) : (0·7812)
Johnny Raw of Hillhouse 1514 (4) : (0·1950)
♂ 2200
White Cockade of Nether Craig 2852 (2) : (0·39)
Cock o' the Walk of Drumjoan 1917 (2) : (0·1462)
♂ App. Vol. 14
Style for Ever of Drumjoan 2362 (5) : (0·4875)
Buntie 3rd of Auchenbrain 9479 (1) : (0·0487)
♂ 4023
Morton Mains Morton Castle 5440 (1) : (0·39)

Diagram 5. *Animals to which inbreeding was traced and which are related to Hover-a-Blink through sire's pedigree.* (*Average milk-producing group*, 1923.)


HOVER-A-BLINK OF DRUMJOAN 892 (8) : (0·7798)
Cock-a-Bendie of Drumjoan 1204 (9) : (0·6334)
Traveller of Drumjoan 1441 (5) : (0·3412)
Traveller's Heir of Holehouse 2903 (5) : (0·9262)
Traveller Again of Holehouse 4561 (2) : (1·1712)
♂ 2392
Sir Thomas of Auchenbrain 2760 (15) : (1·5097)
Prince Robert of Holehouse 4021 (2) : (0·1953)
General MacDonald of Hillhouse 4602 (2) : (0·1950)
♂ 5886
Monkland Zomosal 5887 (2) : (0·5850)
♂ 7027
Brae Rising Star 8187 (3) : (10·9375)
Daniel of Auchenbrain 3585 (3) : (1·1700)
The Sirdar of Auchenbrain 3958 (1) : (0·3900)
Hover's Heir of Southwick 2690 (1) : (0·0975)
♂ 3349
♂ 4486
♂ 4487
Carsegowan John Brown 5070 (6) : (0·9262)
Lessnessock Marshal Oyama 5841 (5) : (1·2675)
Auchenbrain Crusader 6807 (4) : (4·1900)
♂ App. Vol. 19
♀ App. Vol. 22
Desdicado of Overton 4004 (3) : (0·2924)
Not Likely of Hillhouse 4469 (7) : (2·34)
Netherhall Robin Hood 6657 (1) : (0·7800)
♂ 5531
♂ 6486
Lessnessock Gay Scott 7367 (1) : (3·125)
♂ 2690
♂ 3118
Gigantic Stunner of Wyndholm 3872 (1) : (0·0975)
Cockie's Heir of Chapelton 2200 (3) : (0·1949)
White Cockade of Nether Craig 2852 (2) : (0·1462)
Young Cock-a-Bendie App. Vol. 14 (1) : (0·0487)
Style for Ever of Drumjoan 2362 (2) : (0·4387)
Father o' Flynn of Garlaff 1987 (1) : (0·0487)
Asquith of Little Gala 2933 (1) : (0·1950)

Total number of ties for group of animals under 800 gallons for 1923	232
Total percentage of ties traced to animals related to Hover-a-Blink	41·38
Total inbreeding coefficient for group of animals under 800 gallons for 1923... ...	88·69
Total percentage inbreeding coefficient of animals related to Hover-a-Blink	37·33

DISCUSSION.

Homozygosity, *i.e.* purity of characters, can be attained by two methods, namely, by careful systematic selection, or by inbreeding. Every breeder of pedigree stock desires to attain as high a degree of homozygosity in his stock as possible. In dairy breeds the breeder has an absolute guide of the quality or character which he wishes to select, in the actual performance of the animal selected. For a bull, the average milk yield of his daughters, or the average of the combined records of his dam and sire's dam give an excellent indication of his ability to transmit high milk production. For a cow it is possible to take her actual milk record. It is not to be expected therefore that as intensive inbreeding will be carried on in a dairy breed as is done in the beef breeds. In the latter the only judge of quality to guide the breeder lies in what can be seen by eye judgment.

Comparison of the inbreeding of Ayrshire with other breeds.

A comparison of the coefficient of inbreeding of the Clydesdale and of the Beef Shorthorn will clearly show up the two methods. Calder (3) gives the coefficient of inbreeding of the Clydesdale as 6·2 per cent. in 1920–5, and explains how careful and systematic selection without a high degree of inbreeding can bring about homozygosity, even in a breed in which the guide of excellence can only be selected qualitatively by the breeder. The explanation seems to lie in the fact that line breeding, together with careful and continuous selection, has been almost entirely directed to members of one line of descent, viz. that from Darnley. The influence of selection in effecting homozygosity is, according to Calder, also greatly increased by the numerical smallness of the breed, its localised popularity, and by the relatively large number of progeny that one sire is capable of begetting. Wright (1), on the other hand, found the inbreeding coefficient of the Beef Shorthorn to be as high as 25 per cent., a result which indicates that, in a beef breed, type can be most easily fixed by inbreeding.

In the present study, where an easily measurable character, namely milk production, is available to guide the breeder, one would not expect to find... such a high coefficient of inbreeding, since selection will again play a large part in breeding methods. In fact, the Ayrshire breed is shown to be only about 5 per cent. inbred.

Effect of the Drumjoan herd.

The influence of the Drumjoan herd on the Ayrshire breed was very apparent from the beginning of this study. Mr Osborne, the owner, was famous as a bull breeder and exhibitor of Ayrshire bulls. He made great use of Hover-a-Blink, a bull bred by Mr Thomas Hutchison. He also made great use of another bull, Craigs of Kyle, bred by himself. This bull appears as a sire to which inbreeding had occurred seven times in the breed history, and

Total inbreeding coefficient for group of animals under 800 gallons for 1923... ... ... 88·69
Total percentage inbreeding coefficient of animals related to Hover-a-Blink ... 37·33

also appears as the animal with the highest individual number of ties in the pedigrees of the "milk recorded" groups. This being so, it is remarkable that only a very limited number of bulls related to him on the sire's side of the pedigree appear as inbreeding sires in the pedigrees of the "milk recorded" groups. This is altogether different in the case of Hover-a-Blink, whose sons and their descendants figure in almost 50 per cent. of the inbred pedigrees as demonstrated in Table V.

A study of Table IV shows the effective influence of the Drumjoan stock on the high milk producers, the animals giving 1000 gallons and over having double the coefficient of the animals of 800 gallons and under. As late as 1928 bulls of the Drumjoan herd are responsible for the highest coefficient of inbreeding, even though this herd left Drumjoan in 1892. The full significance of the widespread effect of this herd was not realised until the pedigrees of the animals to which inbreeding was traced were themselves fully extended. This also showed the widespread influence of the bull Hover-a-Blink.

Sex-linked inheritance of milking capacity.

The effect of the bull Burnhouses (8) was chiefly expressed through sex-linked inheritance, *i.e.* through his daughter's son White Prince (63) by Lady Flora. On the other hand, Craigs of Kyle (1793) is interesting in that there was only a very limited number of bulls in the "milk recorded" groups traced to him as sire of inbreeding animals, although he himself topped the list for the number of ties causing the inbreeding.

Altogether different is the inheritance of animals related to Hover-a-Blink. This is very clearly demonstrated by the pedigree charts 3, 4 and 5. The manner of his appearance in these pedigrees is contrary to the theory of sex-linked inheritance of milking capacity, but it must be remembered that this bull was famous both for his excellent type and also as a show bull, as were many of his sons. This being the case, he or his male progeny were in demand at a time when the breed was being graded up. The Herd Book still being open for the introduction of animals only twice removed from non-pedigree animals on the female side of the pedigree, and these females being entered on milk record, it is possible that type and constitution were introduced by the bull, and high milk yield by the milk recorded non-pedigree females. In support of this it may be pointed out that two-thirds of the milk record pedigrees trace back, in the second or third generation, to such non-pedigree females on the dam's side of the extended pedigrees.

Comparison of coefficient of breed with the high and average milk-yielding groups.

The "milk recorded" groups are all much less inbred than the average for the breed or for the cows born in the corresponding periods. This has its parallel in Smith's study of the Jersey breed (2). He found that the high milk producers had an average coefficient of only 1·845, as compared with the

breed average of $3{\cdot}913 \pm 0{\cdot}313$, and with the cows of the breed born in that period of $4{\cdot}264 \pm 0{\cdot}456$. It appears from such figures that the policy of breeders for high milk production has been to avoid inbreeding, on the assumption that this was detrimental to milk yield.

That this assumption may be unfounded is shown by the fact that high milk producers of 1000 gallons and over are as inbred as average milk producers of 800 gallons and under.

Comparison of methods of measuring inbreeding.

There is no doubt that Wright's "Long Method"(5) of calculating the coefficient of inbreeding is more suitable for a breed investigation than his "Approximate (or Short) Method." In the former method the complete pedigree of the animal in question is recorded. This gives the investigator an opportunity of studying the manner in which matings have been effected. Briefly it gives the complete genealogy. By the "short method," on the other hand, it is only possible to calculate the degree of inbreeding; the method does not give any opportunity of studying the manner of mating. Its advantage over the "long method" lies in the saving of time in tabulating the pedigrees: thus a larger percentage of the population could be tabulated. Moreover, when pedigrees extend beyond six or seven generations, the use of the "short method" is essential.

It was for these reasons, therefore, that both methods were made use of in the present study. The "short method" was employed for the breed analysis, where the coefficient of inbreeding of the breed was sought. The "long method," on the other hand, was employed in the "milk recorded" groups, since full information was desired on the systems of mating as well as on the inbreeding coefficient.

After the breed history had been completed by the "short method" and in hope of explaining the variations between the percentage of inbreeding of bulls and cows registered in the same year, check samples of the bull and cow populations were taken and analysed. From the results of these checks it became apparent that in a breed of such recent origin as the Ayrshire breed the "short method" is not the best method to employ in a genetic investigation. In this breed as many as two-thirds of the pedigrees only extend to the dam's dam, so that the chances of getting a tie of inbreeding by the "short method" are much less than by the "long method," where the dam's sire can be fully extended and compared with the fully extended pedigree of the animal's sire.

It was only when this study had advanced far enough for comparisons to be made that this aspect of the "short method" as applied to the Ayrshire breed became apparent.

It will be seen from Table III, however, that there is a certain degree of consistency between the results obtained by the two different methods. The breed average coefficient increased approximately 100 per cent. from 1917 to

1922, as also did the cows registered in 1917 and 1922. These results were calculated by the "short method." They have their parallel in the results calculated by the "long method" for the "milk recorded" groups, where the high milk yielders in 1922 also show an approximate 100 per cent. increase in inbreeding over the 1917 group.

Summary.

1. A study has been made of the degree of inbreeding in the Ayrshire breed of cattle.

2. The coefficient of inbreeding for the whole breed, as calculated by Wright's "Approximate (or Short) Method," showed a progressive increase from nil in 1877 to a mean value of 5·3 in 1927.

3. No difference was found in the coefficient of inbreeding as between registered bulls and cows.

4. A large proportion of the inbreeding was traced to two foundation sires, Burnhouses (8) and Hover-a-Blink of Drumjoan (892).

5. Using Wright's "Long Method," it was found that high milk-yielding cows showed a markedly lower coefficient of inbreeding than the breed average. On the other hand, when the coefficient of inbreeding of high milk-yielding cows (over 1000 gallons) was compared with that of cows giving average yields (under 800 gallons) it appeared that inbreeding itself had no detrimental effect on milk yield.

6. In 1923, 39 per cent. of the inbreeding ties of high milk-yielding cows were traced to the Drumjoan herd, as against the next highest herd which had only 6·37 per cent. of ties. Of the total coefficient of inbreeding the Drumjoan herd was responsible for 18·36 per cent., whereas the next highest herd was only responsible for 8·35 per cent. The 1928 results also demonstrated the marked influence of the Drumjoan herd. Practically 50 per cent. of these ties were traced to animals related to the foundation sire, Hover-a-Blink of Drumjoan. These results demonstrate the profound influence which one herd, and indeed one bull, may exert in the genetic construction of a recently established breed.

7. It is pointed out that Wright's "Long Method," when applied to breeds of relatively recent origin, gives far more reliable results than his "Short Method."

Acknowledgments.

The writer takes this opportunity of thanking Prof. F. A. E. Crew for his academic hospitality and for the privilege granted to him of studying at the Institute of Animal Genetics. The writer would also express his gratitude to Mr A. D. Buchanan Smith, to whose helpful advice and encouragement the completion of this study is due, and to Dr N. C. Wright for his advice and criticism in the preparation of the material for publication.

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(*MS. received for publication, August* 20, 1932.)

637.126

46. THE EFFECT OF NINE-, TWELVE- AND FIFTEEN-HOUR INTERVALS DURING THE DAY AND THE NIGHT ON MILK PRODUCTION

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University of Reading.

(With 6 Figures.)

Introduction and object.

During the winter of 1930–1 Campbell(1) carried out work interpreted as showing that under winter conditions night itself, or factors operating at night, apart from the length of the intervals between night and morning milkings, tended to produce morning milk with a lower fat content than the milk produced during the day and drawn at the afternoon milkings.

The following report is based on work carried out from November, 1931, to July, 1932, and on a more extended study of the previous winter's work, to show more clearly that while the length of the interval between milkings does affect the fat percentage, the time of day during which the milk is synthesised is a factor of considerable importance.

Material and method.

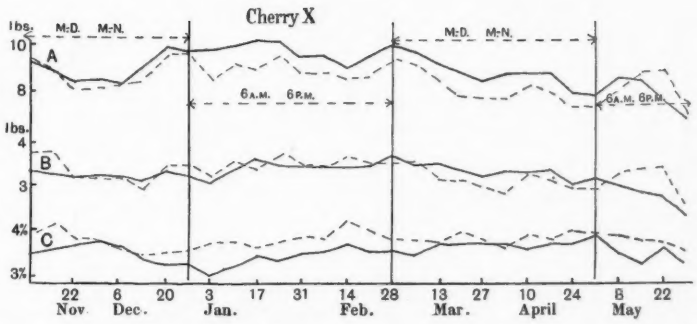
Four cows and two heifers were used in the 1931–2 trial and were introduced into the trial and milked as indicated by the accompanying curves.

Each animal came into the trial within from 4 to 10 days after calving and three of the cows had been used in the trial of 1930–1.

The curves show that the manner of introduction of the cows into the trial would, to some extent, counteract the effect of advancing lactation in so far as average figures are concerned.

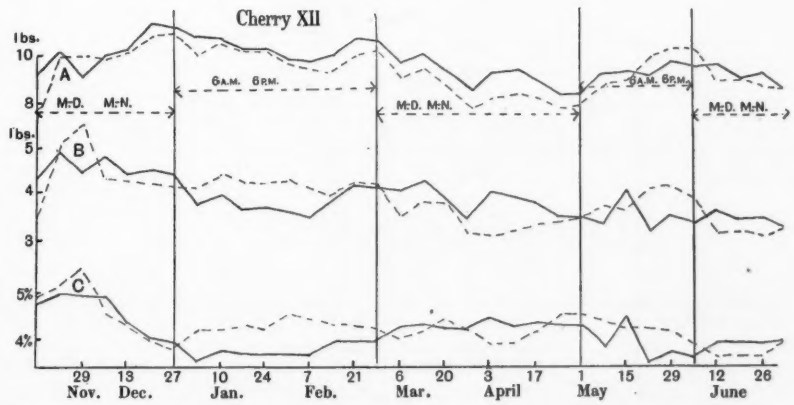
General comment on the curves of individual cows is not necessary except in the case of Rockrose V where the first fortnight in March is omitted. After February 28 this cow was milked at 7 a.m. and 6 p.m. for a special reason before being changed over to midday and midnight milking hours.

The trial started on November 1 with Cherry X; Cherry XII came in on November 9 and Rockrose XIX on November 22. From the beginning of the trial until November 30, the cows were receiving winter keep but were kept in an uncovered yard for the whole of the time between each milking. After November 30, they were put in the yard at about 8.30 a.m. and remained there until the midday milking. After this milking they did not go out again till the following morning.



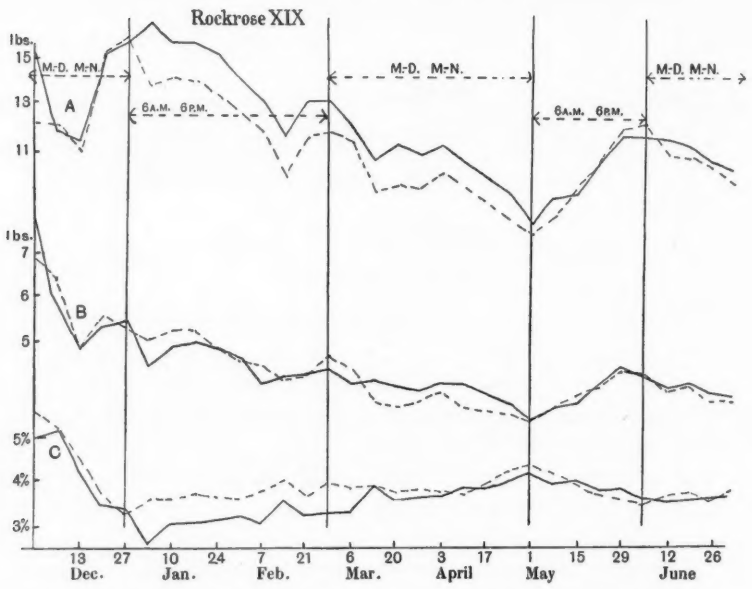
A, —— weight of milk midday and 6 a.m. *B*, —— weight of fat midday and 6 a.m.
---- „ „ midnight and 6 p.m. ---- „ „ midnight and 6 p.m.
C, —— fat per cent. midday and 6 a.m.
---- „ „ midnight and 6 p.m.

Fig. 1.



A, —— weight of milk midday and 6 a.m. *B*, —— weight of fat midday and 6 a.m.
---- „ „ midnight and 6 p.m. ---- „ „ midnight and 6 p.m.
C, —— fat per cent. midday and 6 a.m.
---- „ „ midnight and 6 p.m.

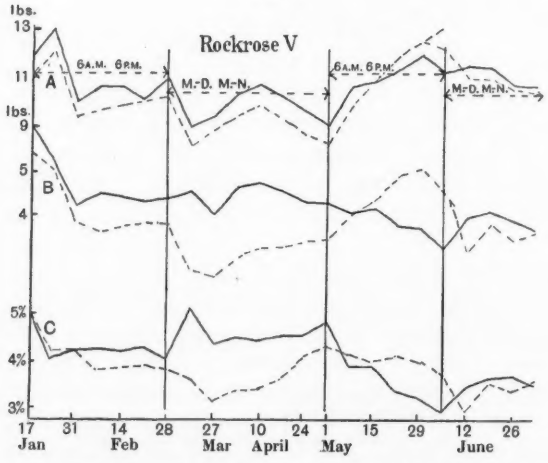
Fig. 2.



A, —— weight of milk midday and 6 a.m. *B*, —— weight of fat midday and 6 a.m.
- - - - „ „ midnight and 6 p.m. - - - - „ „ midnight and 6 p.m.

C, —— fat per cent. midday and 6 a.m.
- - - - „ „ midnight and 6 p.m.

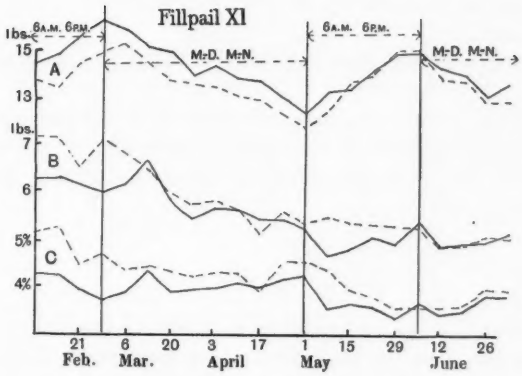
Fig. 3.



A, —— weight of milk 6 a.m. and midday. *B*, —— weight of fat 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight. - - - - „ „ 6 p.m. and midnight.

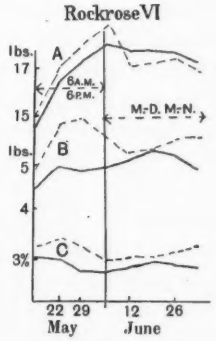
C, —— fat per cent. 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight.

Fig. 4.



A, ——— weight of milk 6 a.m. and midday. *B*, ——— weight of fat 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight. - - - - „ „ 6 p.m. and midnight.
C, ——— fat per cent. 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight.

Fig. 5.



A, ——— weight of milk 6 a.m. and midday. *B*, ——— weight of fat 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight. - - - - „ „ 6 p.m. and midnight.
C, ——— fat per cent. 6 a.m. and midday.
- - - - „ „ 6 p.m. and midnight.

Fig. 6.

After the change over to 6 a.m. and 6 p.m. milking hours, the cows were kept in the yard during the morning but were in the cowhouse for the rest of the day.

On February 8 they went out to grass for a few hours during the day, and on May 1, when they were changed over a second time to 6 a.m. and

6 p.m. milking, they went out to summer keep. For 10 days before this date and before the change over they had been out on grass from after the midday milking till dusk.

Feeding of green food before each milking to supplement grass was started on June 20.

Samples of milk from each cow were taken at each milking and tested for fat by the Gerber method. In the study of these figures, the 6 a.m. milking has been considered to correspond with the midday milking and the 6 p.m. with the midnight.

Results.

Table I shows the yield of milk, yield of fat and true fat per cent. of each cow for each period of the trial. The figures underneath the milk yield and fat yield show those yields expressed as a percentage of the total milk and fat yields respectively at both milkings for that period.

Table II shows the total milk yield and fat yield at 6 a.m. and 6 p.m., both summer and winter, expressed as a percentage for the two milkings per day of those periods. True fat percentages at each milking are also shown. Similar figures are also used for the midday and midnight periods, the winter figures being the result of averaging the milk yield and fat yield for the two periods.

Curves for the individual cows are based on the weekly milk yield, fat yield and fat per cent. at each milking. For instance, Cherry X started the trial at the midday and midnight period. The unbroken lines of the first part of curves *A*, *B* and *C* show the milk yield, fat yield and fat per cent. respectively at the midday milking. The second part of the curves of this cow shows the above yields, in the case of the unbroken lines at 6 a.m. and the broken lines at 6 p.m.

The curves show that during the last three weeks of the second 6 a.m.–6 p.m. period, when the cows were out night and day, the night milk yield was noticeably higher than the morning milk yield. No explanation of this can be offered. On the Wednesday of the third week of this period the cows were put into paddocks near the buildings after the 6 p.m. milking. It must be noticed, however, that all the cows show a higher 6 p.m. yield during the second week and that Rockrose VI, which came into the trial at the beginning of that second week, produced more milk at 6 p.m. than at 6 a.m. from the commencement.

When the cows were changed over to midday and midnight milking hours they were kept in the same paddocks between the midnight and midday milkings as they had been in between the 6 p.m. and 6 a.m. In other words, the grazing during the last period of the trial was the same as that during the period that preceded it, with the following exception. In view of the difficulty of bringing cows along a main road at midnight they were brought from the field at 10 p.m. and kept in the yard until milking time.

Table I.

	Midday			Midnight			6 a.m.			6 p.m.		
	Milk yield	Fat yield	Fat %	Milk yield	Fat yield	Fat %	Milk yield	Fat yield	Fat %	Milk yield	Fat yield	Fat %
	FIRST PERIOD						SECOND PERIOD					
Cherry X	760¾	27·02	3·55	742½	27·99	3·77	877	30·51	3·48	802½	31·05	3·87
	(50·5)	(49·2)		(49·5)	(50·8)		(52·2)	(49·5)		(47·8)	(50·5)	
Cherry XII	716	32·04	4·47	688½	31·03	4·51	931	34·54	3·71	896½	37·89	4·23
	(50·9)	(50·7)		(49·1)	(49·3)		(51)	(47·7)		(49)	(52·3)	
Rockrose XIX	699	29·32	4·19	669¼	28·82	4·31	1289½	40·82	3·16	1139½	42·53	3·73
	(51·1)	(50·3)		(48·9)	(49·7)		(53·1)	(48·9)		(46·9)	(51·1)	
Rockrose V	Not in milk						806¾	34·36	4·26	737¾	30·44	4·12
							(52·2)	(53·0)		(47·8)	(47·0)	
Fillpail XI	Not in milk						657	26·53	4·03	610	29·93	4·90
							(51·9)	(47·0)		(48·1)	(53·0)	
Rockrose VI	Not in milk						Not in milk					
Total	2175¾	88·38	4·06	2100	87·84	4·18	4561½	166·76	3·65	4186	171·84	4·11
*	(50·8)	(50·1)		(49·2)	(49·9)		(52·3)	(49·2)		(47·7)	(50·8)	
†	100	100		96·51	99·38		100	100		91·77	103·04	
	THIRD PERIOD						FOURTH PERIOD					
Cherry X	786½	29·35	3·73	717½	27·72	3·86	316½	10·97	3·46	330¼	12·57	3·80
	(52·2)	(51·3)		(47·8)	(48·7)		(48·9)	(46·6)		(51·1)	(53·4)	
Cherry XII	814½	34·47	4·23	750½	30·84	4·11	469½	17·60	3·75	479½	19·61	4·09
	(52)	(52·8)		(48)	(47·2)		(49·5)	(47·3)		(50·5)	(52·7)	
Rockrose XIX	939¾	34·99	3·72	844	32·72	3·87	524½	19·80	3·78	528½	19·78	3·75
	(52·7)	(51·7)		(47·3)	(48·3)		(49·7)	(50·1)		(50·3)	(49·9)	
Rockrose V	676½	31·37	4·68	622	22·91	3·68	552½	19·38	3·51	568	22·91	4·03
	(52·1)	(57·8)		(47·9)	(42·2)		(49·3)	(45·8)		(50·7)	(54·2)	
Fillpail XI	1279½	51·45	4·02	1214¼	52·32	4·31	708½	24·66	3·49	704¾	26·64	3·79
	(51·3)	(49·6)		(48·7)	(50·4)		(50·1)	(48·2)		(49·9)	(51·8)	
Rockrose VI	Not in milk						661¾	19·09	2·88	685¼	22·22	3·24
							(49·2)	(46·2)		(50·8)	(53·8)	
Total	4496¾	181·64	4·04	4148	166·52	4·01	3232½	111·49	3·44	3296	123·73	3·73
*	(52)	(52·2)		(48)	(47·8)		(49·5)	(47·3)		(50·5)	(52·7)	
†	100	100		92·24	91·67		100	100		101·96	110·97	
	FIFTH PERIOD											
Cherry X	—	—	—	—	—	—						
Cherry XII	363½	14·03	3·87	350	12·76	3·65						
	(50·9)	(52·5)		(49·1)	(47·5)							
Rockrose XIX	436¾	15·60	3·57	415¾	15·22	3·66						
	(51·3)	(50·6)		(48·7)	(49·4)							
Rockrose V	439½	15·88	3·61	426	14·57	3·42						
	(50·7)	(52·1)		(49·3)	(47·9)							
Fillpail XI	548½	19·62	3·58	537¾	19·90	3·70						
	(50·5)	(49·7)		(49·5)	(50·3)							
Rockrose VI	700	20·20	2·88	683½	21·43	3·14						
	(50·6)	(48·4)		(49·4)	(51·6)							
Total	2488¼	85·33	3·43	2413	82·88	3·43						
*	(50·8)	(50·7)		(49·2)	(49·3)							
†	100	100		96·97	97·12							

* Yields at each milking expressed as a percentage of the total yield for each period.
† When the 6 a.m. and midday milk yield and fat yield equal 100, the 6 p.m. and midnight equal as indicated.

Table II.

	Winter periods				Summer periods			
	6 a.m.	6 p.m.	Midday	Midnight	6 a.m.	6 p.m.	Midday	Midnight
Milk yield	52·3	47·7	51·6	48·4	49·5	50·5	50·8	49·2
Fat yield	49·2	50·8	51·5	48·5	47·3	52·7	50·7	49·3
Fat %	3·65	4·11	4·01	4·07	3·44	3·73	3·43	3·43

Discussion.

The curves for individual cows show that milk and fat production did not proceed along the same lines when the milking hours were 6 a.m. and 6 p.m. as when they were midday and midnight. This is true of both winter and summer periods.

The effect of different milking hours showed itself in the fat percentage of the bulk milk at different periods in spite of the method of introduction of the cows into the trial (see Table I).

The average fat per cent. at midday for both winter periods is 4·04, at midnight 4·07.

The average fat per cent. at 6 a.m. (winter period) is 3·65, at 6 p.m. 4·11.

The summer figures are 6 a.m. 3·44, midday 3·43, 6 p.m. 3·73, midnight 3·43.

Correlation with previous results.

In the winter of 1930–1 cows were milked at 6.30 a.m. and 3.30 p.m. for stated periods, and the same cows were milked for other stated periods of the same lactation at 6.30 a.m. and 9.30 p.m. Hence the following comparisons can be made amongst others:

(*a*) Milk production during a 9-hour day and a 9-hour night.

(*b*) Milk production during a 15-hour day and a 15-hour night.

In the winter of 1931–2, cows were milked at 6 a.m. and 6 p.m. for stated periods and then milked at midday and midnight for other stated periods of the same lactations. To the above comparisons can therefore be added the following:

(*c*) Milk production during a 12-hour day and a 12-hour night.

(*d*) Milk production during a 12-hour period, half night and half day, and milk production during a 12-hour period half day and half night.

The production of milk and fat per hour has been calculated for each of the above periods and Table III shows the production per hour during the specified intervals at night when the production per hour during corresponding intervals in the day equals 100.

Table III.

	Length of both day and night intervals	Production per hour during specified day intervals	Production per hour during specified night intervals
Milk	9	100	109·2
	12	100	108·3
	15	100	107·6
Fat	9	100	105·1
	12	100	103·6
	15	100	99·2

Production per hour during 12-hour intervals ending midnight and midday is as follows:

	Ending midnight	Ending midday
Milk	100	101·5
Fat	100	105·3

It is obviously not possible in these trials to produce actual figures showing the fat per cent. of milk after a 9-hour day interval compared with that of the same cows at the same stage of lactation after a 9-hour night.

In the case of the cows milked at 6 a.m. and 6 p.m., however, it can be shown that the actual fat per cent. at 6 p.m. was 4·11, while that at 6 a.m. was 3·65.

Using the figures for a 12-hour day and night interval as shown in Table III and starting with the 6 p.m. fat per cent. of 4·11, the calculated 6 a.m. fat per cent. would be 3·63.

It appears reasonable in view of this agreement to suggest that the figures in Table III are well founded. It is necessary to understand, however, that comparison is made between production per hour during the day by certain cows during certain stages of their lactations with production per hour during the night by the same cows in other stages of their lactations—in the case of both the 9-hour and the 15-hour intervals. In the case of the 12-hour intervals this disadvantage does not apply.

It is clear from the foregoing that under the winter conditions detailed as pertaining to these trials, while both milk and fat production per hour are on a higher level during the night than during the day for intervals as specified, the increased production of milk is greater than the increased production of fat to an extent which results in the milk drawn in the morning having a lower fat content than that drawn in the evening.

Table IV shows the production of milk and fat per hour during a long day and night when that during a short day and night equals 100.

Table IV.

	Production per hour during 9-hour interval	Production per hour during 15-hour interval
Milk: Day	100	92·3
Night	100	91·1
Fat: Day	100	70·7
Night	100	66·7

SUMMARY.

The work herein reported has shown that more milk and fat were produced per hour during 9-, 12- and 15-hour night intervals than during intervals of the same length during the day.

The milk increment was, however, greater than the fat increment.

With midday and midnight milking hours more milk and fat were produced per hour from midnight to midday than from midday to midnight.

In this case the fat increment was the greater.

Conclusions.

Given a night interval of from 9 to 15 hours, under conditions similar to those prevailing in these trials, night itself makes a definite contribution to a low fat percentage in the morning milk.

This will hold true if the night interval exceeds the day interval in length and *vice versa*.

Acknowledgments.

The writer wishes to acknowledge the help of Mr S. Phelp, who milked the cows throughout the trial of 1931–2, and to thank Prof. Pennington for facilities granted for carrying out both trials and Mr D. J. G. Black for laboratory assistance.

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(*MS. received for publication August* 17, 1932.)

636.2:612.664.31

47. THE ERRORS INVOLVED IN CERTAIN METHODS OF ESTIMATING THE LACTATION YIELD OF MILK AND BUTTERFAT

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The many different systems of milk recording in use at the present time are the result of attempts by each country to devise short-cut methods of estimating the lactation yield of milk and butterfat at a minimum cost. A full description of these systems is given in *Dairy Cow Testing in Different Countries* (1).

In Northern Ireland, where a large quantity of milk is sold on a quality basis to creameries for the manufacture of butter, it is imperative that butterfat tests should be made at such intervals as will ensure a reasonable degree of accuracy. The method employed, that of sampling for butterfat at 6-weekly intervals, is not favoured in most other countries. It would seem that an interval of not more than 1 month between butterfat tests is regarded as the most satisfactory compromise between economic considerations and a reasonable degree of accuracy.

As previously reported (2), an investigation was carried out at the Agricultural Research Institute for Northern Ireland, Hillsborough, County Down, between February, 1929, and February, 1930, and in the course of this investigation, records of the milk, butterfat and solids-not-fat, yielded by each cow in the herd at each milking, were obtained. The records of 24 cows, which completed their lactations within the experimental period, are examined in this report.

The object of this analysis of the data is to report on the degree of accuracy of the method used for estimating the lactation yield of milk and butterfat by the Milk Recording Associations in Northern Ireland and to compare this method with other methods.

It should perhaps be pointed out here that the daily variations in the yield and composition of the milk of the herd under investigation are likely to be lower than those found under ordinary commercial conditions. The herd at Hillsborough is milked at the same times each day, even during the week-end, and each milker milks the same group of cows for a period of 1 week and then changes in rotation to another group. The details of management previously noted (2) show that the feeding is carefully controlled. On ordinary

farms it is not always possible, particularly during harvest time and week-ends, to milk at regular intervals and consequently the variations under these conditions may be greater.

Method of investigation.

When the data for each cow at each milking are tabulated, it is possible to select any series of days on which the herd may be assumed to have been visited by a Milk Recording Supervisor, and from each series of days so selected, to obtain a calculated lactation yield of milk and butterfat. For the purpose of this investigation the milk yields were calculated on a weekly and fortnightly basis only. It was thought desirable not to extend the investigation to periods greater than a fortnight as, apart from the lesser degree of accuracy obtained by so doing, much of the value of milk recording is lost, when long periods elapse between the weighings. For weekly weighings all seven possibilities (Monday, Tuesday, etc.) were taken into consideration, and for fortnightly weighings each day of the fourteen was, in the first instance, considered separately. The calculated lactation milk yields were found by multiplying the number of pounds of milk yielded on the days of weighing by the length in days of the interval between the weighings. Seven calculated lactation milk yields for each cow were thus obtained for weekly weighings and fourteen calculated lactation milk yields for fortnightly weighings.

Since butterfat yields are usually calculated on tests made at intervals of much longer duration than is the case with milk yields, a different procedure had to be adopted. To shorten the labour, only one day in each week was considered when calculating the butterfat yield. Wednesday was chosen as the day on which butterfat tests would be least likely to be subject to disturbances, as the change of milkers took place on Mondays. Butterfat yields were therefore calculated on tests made on Wednesdays on a weekly, 2-weekly, 3-weekly, 4-weekly, 6-weekly and 8-weekly basis. Two separate estimations were made for 2-weekly and 3-weekly tests, three for 4-weekly, four for 6-weekly, and five for 8-weekly tests. In order not to confuse the errors involved in calculating milk yields with those involved in calculating butterfat yields, the latter were, in the first instance, calculated from the actual milk yields for the periods. The calculated butterfat yields were therefore found from the formula

$$\Sigma\left(\frac{\text{Actual milk yield} \times \%\text{ butterfat on day of test}}{100}\right),$$

and the calculated butterfat percentages from these results and the actual milk yields.

The errors in calculated milk yields.

The actual milk yields, together with the standard deviations of the errors and the coefficients of variation of the calculated yields, on a weekly and 2-weekly basis, are set out in Table I. It will be seen that there is no con-

nection between the size of the milk yield and the size of the deviation. This is to be expected, as the accuracy of a calculated milk yield depends on:

(*a*) the approximations used when recording the weights;

(*b*) the day-to-day variations in the milk yield;

(*c*) the method of dealing with the broken periods at the beginning and end of the lactation.

In this investigation milk yields were recorded to the nearest quarter-pound, so that the results more nearly approximate to the true yield than the Milk Recording Associations' results, which are based on weighings to the nearest half-pound. The difference, however, is small. The maximum

Table I. *Actual milk yields and standard deviations of errors and coefficients of variation of calculated milk yields.*

Cow No.	Actual yield (lb.)	S.D. of errors on weekly basis (lb.)	S.D. of errors on 2-weekly basis (lb.)	Coefficient of variation (weekly)	Coefficient of variation (2-weekly)
1	7,631¼	70·82	180·14	0·93	2·36
2	8,216½	72·59	165·08	0·88	2·01
3	6,998¾	69·83	137·10	1·00	1·96
4	10,062¼	73·29	114·11	0·73	1·14
5	7,137	106·62	197·05	1·49	2·76
7	10,080¼	85·82	183·37	0·85	1·82
8	8,207	168·87	226·96	2·06	2·77
9	4,165½	63·24	179·88	1·52	4·32
10	11,587½	148·46	229·41	1·28	1·98
11	8,170¾	129·83	194·89	1·59	2·39
13	6,296¾	102·88	222·03	1·63	3·53
16	14,244¾	160·24	236·71	1·13	1·66
17	7,151¼	105·63	210·17	1·48	2·94
18	5,808¼	128·64	253·78	2·21	4·37
21	8,985	90·96	179·15	1·01	1·99
34	7,422¾	217·14	255·94	2·93	3·45
36	6,572½	88·48	148·24	1·35	2·26
37	11,913	172·56	248·87	1·45	2·09
38	8,933	145·38	231·91	1·63	2·60
39	7,668½	124·57	164·33	1·62	2·14
40	3,471½	28·10	70·04	0·81	2·02
41	3,575½	32·96	86·63	0·92	2·42
42	6,336¼	99·72	186·90	1·57	2·95
43	11,968	67·85	141·34	0·57	1·18
Averages	8,025·15	106·40	185·20	1·36	2·46

possible difference over a lactation recorded by 40 weekly weighings would be $\pm 40 \times \frac{1}{4} \times 7 = \pm 70$ lb., roughly less than 1 per cent. of the total yield. That the day-to-day variations in milk yield may have a significant effect on the results will be seen from the fact that between individual cows the variations for weekly weighings range from 0·57 to 2·93, the latter occurring in the case of Cow 34. This cow was noted as "sick" at intervals from 13. vi. 29 to 11. x. 29, and no doubt illness accounted for the variations in the daily milk yield.

The discrepancy due to the odd days not taken into consideration at the beginning and end of the lactation is small. It is the practice in Northern Ireland to record the milk yield even if the cow has calved only 2 or 3 days

before the day of weighing. If the weighing day be regarded as the fourth day of the week in which it occurs, there is a small underestimate (4 days' yield) in the case of a cow calving on the weighing day of the previous week and a small overestimate (2 days' yield) in the case of a cow calving on the day previous to the weighing day. In both cases the errors are offset by the fact that the daily milk yield at this period is usually increasing. At the end of the lactation the error due to the assumption that the lactation period ends on the third day after the last weighing, is negligible. There is no method of avoiding these errors except by daily weighings during these periods, and in view of the smallness of the errors introduced this would appear to be unnecessary.

The average actual yield of the cows under consideration is 8025 lb., and the average standard deviation of the errors is 106·4 lb. Therefore, in round numbers, milk yields of 800 gallons estimated on a weekly basis will differ from the true yields by more than 20 gallons in one estimate out of twenty, while the other nineteen estimates are likely to differ by less than 20 gallons from the true yield. If 20 gallons be regarded as $2\frac{1}{2}$ per cent. of the yield, it would seem that estimating the milk yield on a weekly basis is sufficiently accurate for all practical purposes.

The errors on a 2-weekly basis are about twice those on a weekly basis. Although the average coefficient of variation of 2·46 per cent. is not particularly great, it must be remembered that this error will have an effect upon the error in periodical butterfat testing. This effect is discussed later. The total error might be unduly large if, for the milk yield, one estimate in twenty differed by 5 per cent. or more from the true yield.

The question of the accuracy of estimated milk yields arises in connection with the registration of cows according to milk yield. The average standard deviation of the weekly errors expressed as a percentage is 1·36. When the true milk yield is 6000 lb. it can be shown that in 5 per cent. of the estimates the calculated milk yield may be either below 5837 lb. or above 6163 lb., and that when the true milk yield is 10,000 lb. the calculated milk yield may be either below 9728 lb. or above 10,272 lb. Conversely, when the calculated milk yield is 6000 lb., the true milk yield may in some cases be as low as 5837 lb. It is obvious that some cows are penalised owing to the adherence to a definite standard, and that an approximately equal number of cows are registered, although their true milk yields are below the minimum standard. Any latitude in the case of cows which just fail to reach the prescribed standard would result in practice in a lowering of the standard. It is therefore desirable that a definite standard of production should be rigidly adhered to, in spite of the fact that in some cases cows may be unfairly excluded.

Errors in calculated butterfat yields.

As previously stated, the actual yield of milk for the period covered by the test was taken when estimating the lactation yield of butterfat by means of periodical tests, and only one day in each week (Wednesday) was considered. Altogether calculations were made for six different sets of periodical tests (weekly, 2-weekly, etc.), and seventeen different results for each cow were obtained (Table II).

When the interval between the tests is made longer, the difference between the calculated results for a given interval of testing becomes more marked. Apparently this is due to the fluctuations in the butterfat percentage from day to day and also depends to some extent on the number of tests made during the lactation.

The errors tabulated in Table II are, generally speaking, small. As the interval between the tests is made longer the differences between the calculated results and the actual butterfat tests become more pronounced. It is obvious from a study of the table that some cows are more erratic in butterfat tests than others. In some cases it will be noted that there are exceptionally large divergences from the actual butterfat test. These results are due to the inclusion of occasional abnormally high or low butterfat tests for which it would be difficult to give a satisfactory explanation. It is the practice in the Milk Recording Associations in Northern Ireland to discard such tests, and to resample the cow when an extra visit can be arranged.

Under ideal conditions, the test for butterfat should be made when half the interval has elapsed. In practice, samplers would find it impossible to arrange their visits under these conditions. Further, the element of surprise would be lacking and the practice of visiting a herd at fixed times might lead to abuse of the system. It would appear from the averages of the errors in Table II that the size of the error for any interval is not dependent on when the test is made within the interval. The data show a tendency, however, for tests made towards the end of an interval to vary more often in a positive direction than in a negative direction. This tendency is easily explained when it is remembered that during the greater part of the lactation, the butterfat test is increasing.

A better idea of the extent of the errors may be obtained from a study of Table III in which the errors of the calculated butterfat tests are expressed as percentages of the actual butterfat tests. Here the maximum deviation of the errors rises from 3·13 per cent. for the weekly tests to 12·79 per cent. for the 8-weekly tests. The abnormally high deviation of 19·84 per cent. in the case of Cow 3 was found on investigation to be due to the inclusion of a butterfat percentage of 5·85 per cent. in the early part of the lactation. This table brings out the fact that even the figures for butterfat based on 8-weekly tests may sometimes give results which closely approximate to the true figures. In the majority of cases, however, the divergence of the estimated

Table II. *Errors in calculated butterfat percentages.*

(Calculated percentage—actual percentage.)

Cow No.	Actual butter-fat %	Weekly	2-weekly		3-weekly		4-weekly			6-weekly				8-weekly				
			1st Wed.	2nd Wed.	1st Wed.	3rd Wed.	1st Wed.	2nd Wed.	4th Wed.	1st Wed.	2nd Wed.	5th Wed.	6th Wed.	1st Wed.	2nd Wed.	4th Wed.	7th Wed.	8th Wed.
1	4·26	+0·03	−0·01	+0·07	+0·05	−0·02	+0·12	+0·05	+0·09	+0·09	+0·12	−0·13	+0·08	+0·22	+0·04	−0·05	−0·17	+0·22
2	3·82	+0·02	0	+0·04	+0·04	+0·10	−0·04	−0·01	+0·13	+0·06	−0·12	−0·24	+0·21	−0·03	−0·01	−0·07	+0·16	+0·39
3	3·73	+0·06	+0·19	−0·07	+0·05	−0·04	+0·37	−0·27	+0·14	−0·12	−0·34	+0·74	−0·08	−0·07	−0·43	−0·02	−0·01	+0·27
4	4·19	+0·02	−0·06	+0·10	−0·07	+0·16	−0·08	−0·06	+0·28	+0·08	+0·07	−0·12	+0·52	−0·20	+0·03	+0·52	−0·01	−0·01
5	3·87	−0·04	+0·03	−0·10	−0·13	0	+0·08	−0·02	−0·20	−0·22	−0·17	+0·24	−0·10	−0·01	−0·16	−0·35	+0·23	−0·02
7	3·62	+0·02	+0·01	+0·03	−0·07	+0·13	−0·01	+0·02	+0·02	−0·06	−0·01	+0·04	+0·20	−0·05	−0·01	−0·02	+0·04	+0·07
8	3·97	+0·07	+0·08	+0·06	−0·01	+0·24	+0·05	+0·13	+0·24	−0·01	+0·09	−0·09	+0·12	−0·04	−0·08	+0·50	+0·01	−0·04
9	3·54	+0·01	−0·12	+0·17	+0·09	−0·08	−0·13	+0·18	+0·14	−0·04	+0·17	−0·17	+0·06	−0·16	+0·12	+0·07	−0·06	+0·21
10	3·92	−0·03	−0·03	−0·03	−0·08	+0·04	−0·06	−0·04	−0·02	−0·06	−0·03	−0·07	+0·01	−0·04	−0·15	−0·16	+0·14	+0·12
11	4·17	+0·04	−0·01	+0·09	+0·24	−0·06	−0·01	+0·23	−0·04	+0·04	−0·03	−0·09	−0·17	+0·04	+0·40	−0·21	+0·03	+0·16
13	3·52	+0·05	+0·09	+0·01	−0·04	+0·09	+0·12	+0·09	−0·04	+0·11	+0·32	−0·06	−0·06	+0·33	+0·24	−0·19	+0·09	+0·13
16	3·60	−0·06	−0·03	−0·09	−0·09	+0·09	+0·01	−0·06	−0·11	0	−0·17	−0·17	+0·09	+0·02	−0·05	−0·25	+0·05	+0·03
17	3·71	−0·02	−0·03	−0·03	−0·01	−0·03	+0·02	−0·06	0	+0·05	−0·05	−0·04	+0·07	+0·02	−0·02	−0·10	−0·01	+0·13
18	3·36	−0·02	−0·13	+0·07	+0·20	−0·15	−0·08	−0·08	+0·25	−0·01	−0·14	−0·14	−0·14	−0·21	+0·01	+0·43	−0·11	+0·09
21	3·26	−0·05	−0·04	−0·05	+0·01	−0·03	+0·04	−0·12	+0·02	−0·06	−0·16	−0·09	−0·11	+0·12	−0·08	−0·02	−0·13	+0·07
34	3·91	−0·08	−0·12	−0·04	−0·17	+0·10	−0·16	−0·03	−0·05	−0·29	−0·25	−0·08	+0·19	−0·18	−0·01	−0·03	−0·17	−0·08
36	4·38	0	−0·06	+0·06	+0·05	+0·05	−0·07	+0·11	−0·01	−0·04	−0·16	−0·05	+0·03	+0·02	−0·09	+0·01	+0·06	−0·02
37	3·53	+0·03	+0·08	−0·02	−0·01	+0·05	+0·10	−0·12	+0·07	−0·01	+0·01	+0·07	−0·07	+0·31	−0·13	+0·19	0	−0·06
38	3·85	+0·03	−0·02	+0·09	+0·23	−0·03	+0·11	+0·07	+0·09	+0·12	0	−0·22	−0·09	+0·19	+0·02	+0·30	+0·09	−0·11
39	3·84	−0·12	−0·14	−0·11	−0·07	−0·12	+0·13	−0·14	−0·07	−0·13	−0·28	−0·07	−0·07	−0·02	−0·28	+0·10	−0·16	−0·26
40	4·19	+0·07	+0·06	+0·08	−0·05	+0·22	−0·04	0	+0·16	−0·15	+0·01	+0·09	+0·20	−0·06	−0·03	+0·07	+0·32	+0·26
41	4·20	+0·01	−0·03	+0·09	−0·11	+0·03	−0·09	−0·02	+0·21	−0·14	+0·24	0	+0·11	−0·26	−0·11	+0·09	+0·10	+0·36
42	3·40	+0·06	+0·13	0	+0·12	−0·04	+0·13	−0·02	+0·03	+0·15	−0·02	+0·29	−0·07	+0·26	−0·09	−0·05	+0·14	+0·06
43	3·41	+0·05	0	+0·09	+0·13	+0·05	−0·04	+0·15	+0·03	+0·11	+0·01	−0·08	+0·12	+0·12	+0·17	+0·13	+0·11	−0·06
Average of errors (without signs)		0·04	0·06	0·07	0·09	0·08	0·09	0·09	0·10	0·09	0·12	0·14	0·12	0·12	0·12	0·16	0·10	0·13
No. of errors in positive direction			8	14	11	13	12	9	15	9	9	6	14	10	8	11	14	15

Table III. *Percentage errors in calculated butterfat percentages.*

$$\text{(Calculated test—actual test)} \times \frac{100}{\text{Actual test}}.$$

Cow No.	Actual butter-fat %	Weekly	2-weekly 1st Wed.	2-weekly 2nd Wed.	3-weekly 1st Wed.	3-weekly 3rd Wed.	4-weekly 1st Wed.	4-weekly 2nd Wed.	4-weekly 4th Wed.	6-weekly 1st Wed.	6-weekly 2nd Wed.	6-weekly 5th Wed.	6-weekly 6th Wed.	8-weekly 1st Wed.	8-weekly 2nd Wed.	8-weekly 4th Wed.	8-weekly 7th Wed.	8-weekly 8th Wed.
1	4·26	+0·70	−0·24	+1·64	+1·17	−0·47	+2·81	+1·17	+2·11	+2·11	+2·81	− 3·05	+ 1·88	+5·16	+ 0·93	− 1·17	−3·99	+ 5·16
2	3·82	+0·52	0	+1·04	+1·04	+2·61	−1·04	−0·26	+3·40	+1·57	−3·14	− 6·28	+ 5·50	−0·79	− 0·26	− 1·83	+4·18	+10·18
3	3·73	+1·61	+5·09	−1·88	+1·34	−1·07	+9·92	−7·24	+3·75	−3·22	−9·12	+19·84	− 2·14	−1·93	−11·53	− 0·54	−0·27	+ 7·24
4	4·19	+0·48	−1·43	+2·39	−1·67	+3·82	−1·91	−1·43	+6·68	+1·91	+1·67	− 2·86	+12·41	−4·77	+ 0·72	+12·41	−0·24	− 0·24
5	3·87	−1·03	+0·78	−2·58	−3·36	0	−2·07	−0·52	−5·17	−5·68	−4·39	+ 6·20	− 2·58	−0·26	− 4·13	− 9·04	+5·94	− 0·52
7	3·62	+0·55	+0·28	+0·83	−1·93	+3·59	−0·28	+0·55	+0·55	−1·66	−0·28	+ 1·10	+ 5·52	−1·38	− 0·28	− 0·55	+1·10	+ 1·93
8	3·97	+1·76	+2·02	+1·51	−0·25	+6·05	+1·26	−3·27	+6·05	−0·25	+2·27	− 2·27	+ 3·02	−1·01	− 2·02	+12·59	+0·25	− 1·01
9	3·54	+0·28	−3·39	+4·80	+2·54	−2·26	−3·67	+5·08	+3·95	−1·13	+4·80	− 4·80	+ 1·69	−4·52	+ 3·39	+ 1·98	−1·69	+ 5·93
10	3·92	−0·76	−0·77	−0·77	−2·04	+1·02	−1·53	−1·02	−0·51	−1·53	−0·76	− 1·79	+ 0·26	−1·02	− 3·83	− 4·08	+3·57	+ 3·06
11	4·17	+0·96	−0·24	+2·16	+5·76	−1·44	−0·24	+5·52	−0·96	+0·96	−0·72	− 2·16	− 4·08	+0·96	+ 9·59	− 5·04	+0·72	+ 3·84
13	3·52	+1·42	+2·56	+0·28	−1·14	+2·56	+3·41	+2·56	−1·14	+3·13	+9·09	− 1·70	− 1·70	+9·38	+ 6·82	− 5·40	+2·56	+ 3·69
16	3·60	−1·67	−0·83	−2·50	−2·50	+2·50	+0·28	−1·67	−3·06	0	−4·72	− 4·72	+ 2·50	+0·56	− 1·39	− 6·94	+1·39	+ 0·83
17	3·71	−0·54	−0·81	−0·81	−0·27	−0·81	+0·54	−1·62	0	+1·35	−1·35	− 1·08	+ 1·89	+0·54	− 0·54	− 2·70	−0·27	+ 3·50
18	3·36	−0·60	−3·87	+2·08	+5·95	−4·46	−2·38	−2·38	+7·44	−0·30	−4·17	− 4·17	− 4·17	−6·25	+ 0·30	+12·79	−3·27	+ 2·68
21	3·26	−1·53	−1·23	−1·53	+0·31	−0·92	+1·23	−3·68	+0·61	−1·84	−4·91	− 2·76	− 3·37	+3·68	− 2·45	− 0·61	−3·99	+ 2·15
34	3·91	−2·05	−3·07	−1·02	−4·35	+2·56	−4·09	−0·77	−1·28	−7·42	−6·39	− 2·05	+ 4·86	+4·60	− 0·26	− 0·77	−4·35	− 2·05
36	4·38	0	−1·37	+1·37	+1·14	+1·14	−1·60	+2·51	−0·23	−0·91	−3·65	− 1·14	+ 0·69	+0·46	− 2·05	+ 0·23	+1·37	− 0·46
37	3·53	+0·85	+2·27	−0·57	−0·28	+1·42	+2·83	−3·40	+1·98	−0·28	+0·28	+ 1·98	− 1·98	+8·78	− 3·68	+ 5·38	0	− 1·70
38	3·85	+0·78	−0·52	+2·34	+5·97	−0·78	+2·86	+1·82	+2·34	+3·12	0	− 5·71	− 2·34	+4·94	+ 0·52	+ 7·79	+2·34	− 2·86
39	3·84	−3·13	−3·65	−2·86	−1·82	−3·13	+3·39	−3·65	−1·82	−3·39	−7·29	− 1·82	− 1·82	−0·52	− 7·29	+ 2·60	−4·17	− 6·77
40	4·19	+1·67	+1·43	+1·91	−1·19	+5·25	−0·95	0	+3·82	−3·58	+0·24	+ 2·15	+ 4·77	−1·43	− 0·72	+ 1·67	+7·64	+ 6·21
41	4·20	+0·24	−0·71	+2·14	−2·62	+0·71	−2·14	−0·48	+5·00	−3·33	+5·71	0	+ 2·62	−6·19	− 2·62	+ 2·14	+2·38	+ 8·57
42	3·40	+1·76	+3·82	0	+3·53	−1·18	+3·62	−0·59	+0·88	+4·41	−0·59	+ 8·53	− 2·06	+7·65	− 2·65	− 1·47	+4·12	+ 1·76
43	3·41	+1·47	0	+2·64	+3·81	+1·47	−1·18	+4·40	+0·88	+3·23	+0·29	− 2·35	+ 3·52	+3·52	+ 4·99	+ 3·81	+3·23	− 1·76
Maximum error (%)		3·13	5·09		6·05		9·92			12·41 (19·84)				12·79				

butterfat figures, based on 8-weekly intervals, from the true figures is considerable.

Table IV shows the average butterfat percentage of the herd for the different estimations. The greatest difference between the actual average and a calculated average is 0·06 per cent. It is not to be concluded, however, that results of this nature would be obtained in individual cases. All that this table shows is that the errors in individual cases tend to cancel out, and that if only the butterfat production of a herd is required, 8-weekly tests of individual cows or of a properly prepared composite sample of one day's milk of the herd would give a good approximation.

Table IV. *Calculated average percentages butterfat for herd for different intervals.*

(Actual average percentage of butterfat for herd = 3·78.)

Calculated on weekly basis	Calculated on 2-weekly basis	Calculated on 3-weekly basis	Calculated on 4-weekly basis	Calculated on 6-weekly basis	Calculated on 8-weekly basis
3·78 (1)	3·77 (1)	3·79 (1)	3·80 (1)	3·76 (1)	3·81 (1)
—	3·79 (2)	3·81 (3)	3·76 (2)	3·73 (2)	3·75 (2)
—	—	—	3·82 (4)	3·75 (5)	3·81 (4)
—	—	—	—	3·83 (6)	3·81 (7)
—	—	—	—	—	3·84 (8)
		Mean of all tests for each interval			
3·78	3·78	3·80	3·79	3·77	3·80

Figures in brackets show on which Wednesday in the intervals the averages were calculated.

The standard deviation of the errors of the herd increases markedly as the interval between the tests is made longer (Table V). This table brings out the fact that the estimations of individual lactation yields are distributed over a much wider range than would appear from the averages of the calculated tests. For example, for 6-weekly tests, one estimate in twenty will differ from the true estimate by 8·58 per cent. or more of the actual test. On the other hand, weekly tests give a very close approximation to the true test.

Table V. *Standard deviations of errors in calculated average percentage butterfat for different intervals.*

	Weekly	2-weekly	3-weekly	4-weekly	6-weekly	8-weekly
	0·050	0·083	0·112	0·114	0·113	0·163
	—	0·075	0·099	0·113	0·160	0·164
	—	—	—	0·120	0·205	0·224
	—	—	—	—	0·154	0·125
	—	—	—	—	—	0·155
s.d. all tests	0·050	0·079	0·105	0·117	0·162	0·170
			Coefficients of variation			
	1·32	2·20	2·96	3·02	2·99	4·31
	—	1·98	2·62	2·99	4·23	4·34
	—	—	—	3·17	5·42	5·93
	—	—	—	—	4·07	3·31
	—	—	—	—	—	4·10
All tests	1·32	2·09	2·78	3·10	4·29	4·50

DISCUSSION OF RESULTS.

In the case of the milk yields, there is no difficulty in defining the limits within which weighings may be made. Weekly weighings are an established practice, and it is shown here that the results obtained agree closely with the actual figures. It is pointed out also that a certain amount of accuracy is sacrificed when the weighings are made fortnightly and that there is no particular advantage in curtailing the weighings to this extent.

Owing to the greater degree of variability of the butterfat, the longer interval between the tests, and the limited number of observations for each interval, it is more difficult to deal with the calculated butterfat tests. There is, on the one hand, the fact that the possible error, even in the case of weekly tests, is significant, and on the other, the fact that economic considerations render some form of short-cut method essential.

A further complication is that under practical conditions any errors arising out of periodical butterfat testing are superimposed on a calculated milk yield which in itself is subject to error, with the result that the possible error

Table VI. *Comparison of errors in butterfat percentages when the results are based on the actual milk yield and on the milk yield estimated on a weekly basis.*

	Based on actual milk yield		Based on calculated milk yield	
	Max. error (% butterfat)	S.D. of errors (% butterfat)	Max. error (% butterfat)	S.D. of errors (% butterfat)
4-weekly tests	0·37	0·117	0·37	0·108
6-weekly tests	0·34 (0·74)*	0·162	0·53	0·162
8-weekly tests	0·52	0·170	0·53 (0·84)*	0·173

* The figures in brackets indicate what the maximum error would have been if the abnormal test of Cow 3 had been included.

in the calculated butterfat yield, due to the two processes, may in some cases be increased. This will occur when the two errors are in the same direction. On the other hand, when the two errors are of opposite sign, the calculated butterfat yield may be close to the actual butterfat yield. At first sight, therefore, it would seem that the effect of combining periodical butterfat testing with calculated instead of actual milk yields, would be to distribute the errors over a wider range. It was thought advisable to prepare a further set of results, basing the calculations on weekly weighings and tests made at intervals of 4, 6 and 8 weeks. These results are set out in Table VI.

Table VI shows that the standard deviations of the errors in the calculated butterfat percentages based on weekly weighings are not appreciably greater than those based on actual milk yields. The range of the errors is, therefore, not much increased when the calculated milk yield is used instead of the actual milk yield. This presumably is explained by the fact that the errors in the calculated milk yields are small when compared with the errors in the calculated butterfat percentages.

On the assumption that 95 per cent. of the errors will be less than twice the standard deviation, it will be seen from Table VI that most of the errors in the case of 4-weekly tests will be less than ± 0·22 per cent. of butterfat. For 6-weekly tests this figure becomes ± 0·32 per cent. and for 8-weekly tests ± 0·35 per cent. Some errors will be found to exceed these limits. (It does not follow that the maximum errors found in this particular investigation indicate the greatest possible errors.)

Expressing twice the standard deviation as a percentage of the true average test (3·78 per cent.) the above limits become: for 4-weekly tests 5·82 per cent., for 6-weekly tests 8·47 per cent. and for 8-weekly tests 9·26 per cent. In view of the fact that the maximum errors noted in Table VI are over three times the standard deviations of the errors, it will be seen that to ensure that the errors be less than 10 per cent., it would be necessary to test for butterfat at intervals of 4 weeks. With 6-weekly or 8-weekly intervals between the tests, a certain number of errors would exceed this limit. (If it is required to have a butterfat percentage accurate to within 5 per cent., the tests would have to be made fortnightly.)

It is hardly necessary to point out that when the average butterfat percentage of a large number of cows is under consideration, these errors will tend to cancel out. For this reason the average butterfat percentage of all the cows under test in Northern Ireland does not vary, or varies only a little, from year to year. For the same reason, tests made at creameries, where the mixed milk of herds is tested, are more uniform than milk-recording tests.

The results obtained in this investigation leave no doubt as to the desirability of testing for butterfat at frequent intervals. The shortening of the interval between butterfat tests would, however, involve a considerable amount of extra work and expense. If 4-weekly intervals were adopted in Northern Ireland, the number of visits to each farm would be increased by 50 per cent., and in some districts, owing to the small number of cows per herd, supervisors could not visit 150 cows in a month.

Summary.

An analysis of the data collected at the Agricultural Research Institute for Northern Ireland, Hillsborough, County Down, was undertaken to find if the system of milk recording practised in Northern Ireland is satisfactory.

The standard deviations of the errors in the calculated milk yields, on a weekly and 2-weekly basis, of 24 cows were found. It is shown that weekly weighings give results which approximate closely to the true milk yield.

By basing the calculated butterfat yields on tests made at intervals of from 1 to 8 weeks, the standard deviations of the errors in the calculated butterfat percentages for each interval, as well as the maximum errors, were found. It is shown that as the interval between the tests is lengthened, the errors increase. With tests made at intervals of 6 weeks, most of the errors

in the calculated butterfat percentages will be less than 8·5 per cent., but in some cases the calculated butterfat percentage may differ from the true butterfat percentage by over 12 per cent.

In order to ensure that the errors in calculated butterfat percentages do not exceed 10 per cent., the interval between butterfat tests should not exceed 1 month.

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(*MS. received for publication July* 6, 1932.)

637.1:543/545

48. INVESTIGATIONS ON THE MILK OF A TYPICAL HERD OF SHORTHORN COWS. I

By J. GOLDING, J. MACKINTOSH and E. C. V. MATTICK.

(With 1 Figure.)

During the summer of 1928 the staff of this Institute decided to carry out an investigation into the composition of milk as produced under typical south of England conditions. The work is still in progress, but the following report deals with the three-year period October 1, 1928, to September 30, 1931, and gives a description of the methods used and a summary of the main results obtained.

Method.

For this investigation ten dairy Shorthorn cows of varying ages and calving at different times of the year were chosen to constitute a typical small herd, and from October 1, 1928, onwards the management and feeding have been on uniform lines, resembling the common practice in a well-managed herd in the south of England. Details of the cows, milk yields, dates of calving and feeding are given below.

Selection of cows.

The ten cows chosen in 1928 had all been reared on the Institute's farm (nine were home-bred) under similar conditions, and the ages ranged from 3 years to about 7 years. During the first year of the investigation two cows were in their fifth lactation period, one in her fourth, two in their third, four in their second, and one in her first lactation. Four of the ten were the daughters of one sire; four were by another bull and the remaining two were by two different sires. All the sires were registered Dairy Shorthorn Bulls and the ten cows represented all stages of the grading-up process according to the Dairy Shorthorn Association's scheme: one was in class A; two in class B; one in class C; three in class D; and two were entered in Coates' Herd Book.

In April, 1929, one cow was accidentally strangled in the cowshed, and in October, 1930, another, which had lost two quarters by an attack of mastitis previous to calving, was sold fat. In November, 1929, one home-bred heifer was added to the herd but she proved a most disappointing milker and was fattened and sold early in 1930. In September and in December, 1930, two more home-bred heifers were introduced to make up the numbers; both proved very moderate milkers and one was sold in September, 1931.

HEALTH.

The general health of all the cows has been very good throughout the three-year period. The only serious disturbance was an attack of "husk" in June and July, 1930, which affected almost all the cows on the farm. Of the cows in this group Rosebud suffered most; she lost condition rapidly and dried off after a comparatively short lactation period of 232 days. Ultimately she made a good recovery. Doll 2nd suffered from milk fever at calving in March, 1930, and was separated from the other cows for several weeks. Rosebud calved a dead calf in December, 1930. All other calvings were normal, and throughout the lactation periods there have been very few instances of udder trouble.

MANAGEMENT.

A good standard of management has been maintained throughout the three years. During the winter from late October to April, the cows have been out for a few hours during the daytime, except in very rough weather, and housed at night; from April to late October they have been out in the pasture day and night. Dry cows due to calve during the winter have as a rule been out day and night until close to calving.

FEEDING.

Bulky foods and pasture. During the autumn and winter the maintenance part of the ration consisted of meadow hay with cabbage, marrow-stem kale and mangels in succession as the season advanced. During the summer, pasture constituted the sole food as far as possible, supplemented when necessary by green forage crops. The weather conditions naturally controlled the supply of grass, and the three years covered by the investigation showed great variation.

1928–9. From October 1, 1928, meadow hay formed part of the ration until May 3, of the next year; cabbage was given from October 1 to 17; kale from October 18 to November 14 and mangels from November 15 to May 3. The summer of 1929 was exceptionally dry and forage crops, etc., had to be freely used; during April there was a gradually increasing amount of pasture, but the full grazing season only extended from May 3 to June 19; from June 19 to 27 green lucerne was given daily; from June 28 to July 7 green vetches and oats took the place of lucerne and from July 8 to 22 a fairly good growth of meadow aftermath made the use of forage crops unnecessary; from July 23 to September 7, however, the barrenness of the pasture and the lack of green forage crops made it necessary to give silage (oat and vetch) daily; on September 8 the giving of marrow-stem kale was begun and continued until October 4, while a small allowance of hay was given from September 11 onwards.

1929–30. Meadow hay was given from September 11, 1929 throughout the winter until April 25, 1930, cabbage from October 4 to November 29 in 1929, and mangels from November 30, 1929 to April 25 in the following year. Pasture was available in increasing amount during April and was abundant until towards the end of June. The normal summer grazing routine had then to be departed from because of the outbreak of "husk." The cows in the typical herd had to be removed from their usual pasture and confined to a smaller field where it was necessary to give green oats and vetches from June 28 to July 6, and hay from July 7 to 26; from July 27 to October 2 green lucerne was given daily in addition to the grazing of a rather short pasture.

1930–1. From October 3 to November 16, 1930, cabbage was given to supplement the pasture, and the use of meadow hay as part of the winter ration did not begin until November 2, from which date it was continued until April 30, 1931. Kale was given from November 17 to December 22 and mangels from December 23, 1930 to April 30, 1931. Pasture became available in increasing amounts during April and continued abundant until September 6—a marked contrast to the two previous seasons—after which date it was supplemented by a moderate allowance of green lucerne until September 29. On September 30 the use of cabbage was recommenced.

The periods when the different bulky foods were given are shown diagrammatically in Table I.

The daily quantities of the above foods varied from time to time, and as it is not possible to control the amount of green foods eaten by individual cows when these foods are spread on the pasture, the following amounts are at best but estimates: green lucerne and green vetches and oats, 20 lb. up to 50 lb. daily in 1930; silage, 33–35 lb. in 1930; cabbage and kale 30–56 lb. daily according to the amount of pasture available. The weights of hay and mangels given indoors ranged from 7 to 14 lb. hay and 40–60 lb. mangels daily according to the size of the cow and the quantity of milk yielded.

Concentrates. The concentrates given during the first winter of the investigation consisted of a mixture of three parts dried brewer's grains, two parts maize-germ meal and one part extracted soya-bean meal—all parts by weight. No home-grown cereals were included because a sufficient supply was not available. For the second and third winters the same foods were also used. The amount given was $3\frac{1}{2}$–$3\frac{3}{4}$ lb. per gallon of milk.

The average analysis of the three foods used and the average composition of the mixtures given to the cows during the winter were as follows:

	Crude protein %	Oil %	Carbo-hydrates %	Fibre %	Ash %	Moisture %
Dried brewers' grains	18·11	7·52	46·60	16·64	3·70	7·44
Maize-germ meal	10·99	7·61	65·07	2·60	2·56	11·16
Soya-bean meal	43·88	0·61	32·93	4·75	6·00	11·82
Mixture	19–21	6–7	50–52	9–11	3·6–3·9	9–11

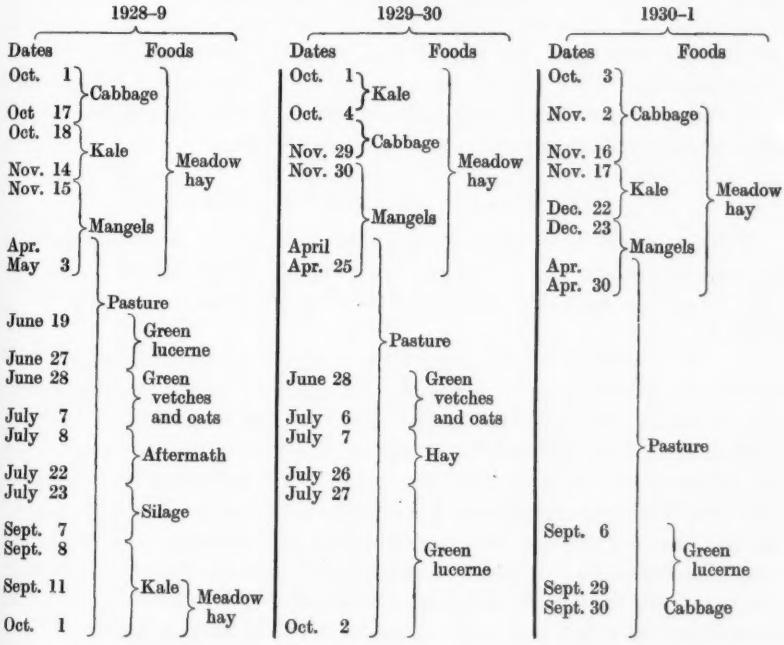
The calculated starch equivalent of the mixture was 61 and the percentage

of protein equivalent was 15·7, hence 3½ lb. of the mixture supplied 2·1 lb. starch equivalent and 0·55 lb. protein equivalent, and 3¾ lb. supplied 2·3 lb. and 0·59 lb. respectively.

The large proportion of dried brewers' grains made the mixture bulky and it has always proved palatable. Almost without exception during the three successive winters all the cows have cleared up their allowance for each feed readily and have shown no signs of tiring of the mixture.

During that part of the summer when good grazing was available soya-bean meal was omitted and the mixture usually consisted of equal weights

Table I. *Showing bulky foods and periods of use from October* 1, 1928 *to September* 30, 1931.



dried grains and maize-germ meal; as the summer advanced soya-bean meal was introduced, the proportions usually being two parts dried grains, two parts maize-germ meal and a half part soya-bean meal. In May the concentrate mixture was given to the cows yielding more than 3–4 gallons daily at the rate of 3 lb. for each extra gallon, and as the season advanced the allowance of concentrates was controlled according to the estimated feeding value of the pasture and green fodder crops given. In 1930 from June 19 to August 30, during the period of the husk attack, the above mixture was replaced by a compound cake of high carbohydrate and low protein content, in order to give a food which was less dusty and perhaps more readily eaten.

Rock salt was placed in the mangers and in the pastures from time to time, but no other minerals were added to the diet or supplied in block form.

Cows in-calf received, for 4–6 weeks previous to calving, an allowance of the mixture given to the cows in milk during the same period.

Milking.

All the cows were milked by reliable and efficient hand milkers throughout the period. The morning milking was carried out from 6.45 to 8.0 a.m. and the afternoon milking from 3.45 to 4.45 p.m. Cows in full milk were usually milked last at night and first in the morning, therefore the above-mentioned milking hours give a night interval of 14½–15½ hours and a day interval of 9½–8½ hours. With a few exceptions each cow was milked by the same milker throughout each lactation period. The milk yielded by each cow at each milking was weighed to the nearest ¼ lb. on a spring balance; the milking pails used were "tared" to suit the balance; the accuracy of the balance was checked from time to time. The milk records were kept and checked under the supervision of the Berkshire Milk Recording Society.

Milk yields and regularity of calvings.

The cows selected to constitute this small herd have on the whole proved satisfactory milkers. The details of calving dates, milk yields and number of days in milk in each of the three years of the investigation are set out in Table II.

It will be seen in this table that several of the cows have calved at approximately yearly intervals, *e.g.* Rosebud, Lottie 2nd and Flora, whereas others have calved at wider intervals, *e.g.* Doll 2nd, Pearl and Winsome 3rd. Doll 2nd suffered from milk fever after calving on March 12, 1930, and was under special treatment for some time; Winsome 3rd has had twins twice in three years and therefore some increase in the interval between calvings is excusable. We are unable at present to say whether the successive dates of calving in this group of cows over a period of three years represents the normal state of affairs in herds managed under similar conditions. It is known that as a rule cows which have had four to seven calves, have an average length of time between calvings of more than 12 months—probably more frequently an interval of 13–14 months—and in this instance the average of 17 intervals is 394 days or 13 months.

Regularity of milk output.

The regularity of the output of milk from a herd depends mainly on the times at which the cows calve. At the beginning of this investigation we were compelled to select cows which calved between September, 1928 and March, 1929, hence the milk yield was at its maximum in March and April and fell fairly rapidly to its minimum for the year in August, when the output was only 36 per cent. of the April yield. In 1929–30 the output during the winter

Table II. *Typical herd. Milk production*, 1928–31.

Cow	Oct. 1, 1928–9, 1st year					Oct. 1, 1929–30, 2nd year					Oct. 1, 1930–1, 3rd year				
	Dates of calving	Lactation period	Yield of milk lb.	Days in milk	Fat content %	Dates of calving	Lactation period	Yield of milk lb.	Days in milk	Fat content %	Dates of calving	Lactation period	Yield of milk lb.	Days in milk	Fat content %
Doll 2nd (born 5. xi. 21)	Dec. 23, 1928	5	11,723½	278	4·09	Mar. 12, 1930	6	6,235	239	3·88	May 22, 1931	7	7,238½	244	3·84
Flora 1st (born 28. xi. 21)	Sept. 27, 1928 Sept. 4, 1929	5 6	12,942	302	3·53	—	6	10,897¼	299	3·24	Oct. 21, 1930	7	12,852¼	288	3·57
Pearl 1st (born 17. iii. 23)	Feb. 19, 1929	4	9,075¼	252	3·98	Feb. 5, 1930	5	10,347	288	3·88	May 28, 1931	6	8,264	252	3·96
Rosebud (born 3. xii. 23)	Dec. 3, 1928	3	8,645½	309	3·93	Nov. 17, 1929	4	8,525	232	3·80	Dec. 6, 1930	5	8,688¾	291	3·66
White Rose (born 4. i. 24)	Sept. 17, 1928 Aug. 24, 1929	3 4	10,822¾	306	3·76	—	4	9,258¼	365	3·65	Sold fat Lost two quarters				
Doris (born 3. x. 24)	Sept. 13, 1928	2	7,454¾	208	3·98	Accidentally killed Apr. 27, 1929									
Winsome 3rd (born 14. x. 24)	Oct. 16, 1928	(Twins) 2	6,913	281	3·60	Feb. 21, 1930	3	7,143	218	3·65	Feb. 25, 1931	(Twins) 4	8,881¼	302	3·56
Lottie 2nd (born 2. iii. 25)	Mar. 17, 1929	2	8,088¾	263	3·44	Mar. 13, 1930	3	7,976¼	259	3·26	Mar. 24, 1931	4	7,738¾	254	3·22
Peach 2nd (born 3. iv. 25)	Jan. 26, 1929	2	9,494¾	298	3·47	Mar. 22, 1930	3	7,776	236	3·92	Mar. 11, 1931	4	9,014¾	295	4·02
Firefly 3rd (born 24. ix. 25)	Aug. 21, 1928 Oct. 4, 1929	1 2	8,391½	321	3·95	—	2	8,636	348	3·89	Oct. 29, 1930	3	7,881½	333	3·85
	Average for 10 cows		9,355			Average for 9 cows		8,532			Average for 8 cows		8,820		
Lucy 3rd (born 9. iv. 27)	—	—	—	—	—	Nov. 7, 1929	1	1,534¼	84	—	Sold fat				
Lottie 3rd (born 16. ii. 28)	—	—	—	—	—	—	—	—	—	—	Sept. 19, 1930 Sept. 3, 1931	1 2	3,968¾	267	3·46
Pauline 2nd (born 19. x. 28)	—	—	—	—	—	—	—	—	—	—	Dec. 15, 1930	1	4,469½	261	3·97

months was lower than in the preceding year, but the maximum yield again occurred during April and the minimum yield in September, when the output was about 47 per cent. of the April yield. In 1930–1 a more uniform output was achieved, the maximum being in June, 1931 and the minimum in October, 1930, when the output was about 45 per cent. of the June, 1931, yield. The greater uniformity of milk yield during 1930–1 was due partly to several of the cows calving later in the season and partly to the greater supply of pasture throughout the summer.

The total milk yield, evening and morning, for each week of the three-year period is shown in Tables VII, VIII and IX.

Sampling and testing.

Throughout the three-year period under review, separate samples were taken from the milk of each cow at each milking. This was done after the milk had been weighed and mixed by pouring from the milking pail into the bucket and back again. It was also stirred with the dipper used for taking the sample.

During the years 1928–9, the milk from each individual cow was separately tested for fat and specific gravity, but these results are not included in the present report.

Composite samples of each morning's and of each evening's milk, were always made up in the laboratory by taking aliquot parts (usually 3 ml. per lb.) from each well-mixed sample.

At intervals, when this "type" milk was required for butter-making, or other researches, the milk from all the cows was bulked in a cheese vat on arriving at the dairy. In such cases further samples were taken from the well-mixed bulked milk, in addition to composite samples made up as usual. This served as a check on the mixing of the bulk samples. The daily routine was limited to the determination of fat, total solids and specific gravity.

The total solids were always determined in duplicate, by evaporating about 5 g. of milk in a flat nickel dish, and drying in a steam oven till the weight was constant. The fat was always determined by the Gerber method, duplicate estimations being made, the results were checked from time to time by a gravimetric method. All the glassware was standardised, and the duplicate fat determinations agreed to within 0·05 per cent. If this difference was exceeded, another determination was made.

The specific gravity was determined at 60° F. (15·5° C.), with a standardised lactometer, the night's milk being tested the following morning, whilst the morning's milk was tested when only a few hours old, except at the week-ends and holidays when samples were sometimes kept for a longer period.

RESULTS.

The separate daily figures for the content of the morning's milk and evening's milk in fat and solids-not-fat are grouped in Tables III, IV, V and VI. In Tables III and IV, a fat percentage distribution is shown for each of the three years under discussion and also for the whole period.

Table III. *Percentage butterfat distribution in daily composite samples of morning's milk.*

Butterfat %	Oct. 2, 1928 to Sept. 30, 1929 1st year	Oct. 1, 1929 to Sept. 30, 1930 2nd year	Oct. 1, 1930 to Sept. 30, 1931 3rd year	Total 3 years
2·4	—	0·56	—	0·19
2·5	—	0·28	—	0·09
2·6	0·56	1·96	0·55	1·02
2·7	—	4·48	0·83	1·77
2·8	3·11	8·12	4·68	5·31
2·9	3·39	3·36	9·09	5·31
3·0	7·06	6·44	14·88	9·50
3·1	14·41	11·49	14·60	13·50
3·2	17·51	16·53	14·05	16·01
3·3	14·41	12·89	10·19	12·48
3·4	16·95	14·57	8·26	13·22
3·5	11·30	8·96	7·99	9·40
3·6	5·93	5·88	5·51	5·77
3·7	3·11	2·80	4·96	3·63
3·8	1·98	0·56	2·48	1·68
3·9	—	0·84	1·10	0·65
4·0	0·28	0·28	0·83	0·47
Total	100·00	100·00	100·00	100·00
Samples tested	354	357	363	1074

Table IV. *Percentage of butterfat distribution in evening's milk.*

Butterfat %	Oct. 1, 1928 to Sept. 30, 1929	Oct. 1, 1929 to Sept. 30, 1930	Oct. 1, 1930 to Sept. 30, 1931	Total
3·6	—	—	0·27	0·09
3·7	—	—	0·27	0·09
3·8	0·29	0·84	0·82	0·66
3·9	0·88	0·56	0·82	0·75
4·0	1·18	1·96	4·66	2·64
4·1	1·18	6·16	5·75	4·43
4·2	4·13	10·09	10·96	8·48
4·3	7·38	9·52	15·07	10·75
4·4	6·79	13·73	11·51	10·75
4·5	10·33	14·29	14·25	13·01
4·6	11·80	7·84	9·32	9·62
4·7	14·16	8·97	8·49	10·47
4·8	13·27	5·04	6·03	8·01
4·9	7·67	6·44	4·39	6·13
5·0	8·85	5·04	2·47	5·37
5·1	5·61	2·24	3·01	3·58
5·2	3·83	2·80	1·37	2·64
5·3	1·18	1·40	—	0·85
5·4	0·59	1·68	0·27	0·85
5·5	0·29	0·56	0·27	0·37
5·6	0·59	0·56	—	0·37
5·7	—	—	—	—
5·8	—	0·28	—	0·09
Total	100·00	100·00	100·00	100·00
Samples tested	339	357	365	1061

From Table III it will be seen that 13·69 per cent. of the analyses for butterfat in the 1074 samples of morning's milk fell below 3 per cent. Also that 16·01 per cent. of the samples contained 3·2 per cent. This is the highest class centre for the three-year period.

The figures varied slightly from year to year. The results of the percentage distribution of the fat in the evening's milk (Table IV) are in marked contrast to those of the morning's milk (Table III). No evening samples contained

Table V. *Percentage solids-not-fat distribution in morning's milk.*

Solids-not-fat %	Oct. 2, 1928 to Sept. 30, 1929	Oct. 1, 1929 to Sept. 30, 1930	Oct. 1, 1930 to Sept. 30, 1931	Total
8·3	—	0·28	—	0·09
8·4	—	0·84	—	0·28
8·5	—	7·00	—	2·33
8·6	—	4·48	—	1·49
8·7	4·24	8·68	0·55	4·47
8·8	13·84	19·89	4·96	12·85
8·9	26·56	25·49	29·20	27·10
9·0	37·01	18·49	42·70	32·77
9·1	11·58	10·93	17·36	13·31
9·2	3·95	3·64	4·41	4·00
9·3	2·26	0·28	0·82	1·12
9·4	0·56	—	—	0·19
Total	100·00	100·00	100·00	100·00
Samples tested	354	357	363	1074

Table VI. *Percentage solids-not-fat distribution in evening's milk.*

Solids-not-fat %	Oct. 1, 1928 to Sept. 30, 1929	Oct. 1, 1929 to Sept. 30, 1930	Oct. 1, 1930 to Sept. 30, 1931	Total
8·3	—	0·56	—	0·18
8·4	0·29	6·44	—	2·26
8·5	0·88	8·68	0·27	3·30
8·6	2·36	7·00	2·47	3·96
8·7	18·29	14·29	10·96	14·42
8·8	31·86	21·01	27·95	26·87
8·9	29·50	22·41	34·52	28·84
9·0	11·51	14·85	20·82	15·84
9·1	4·13	4·20	3·01	3·77
9·2	0·89	0·56	—	0·47
9·3	0·29	—	—	0·09
9·4	—	—	—	—
Total	100·00	100·00	100·00	100·00
Samples tested	339	357	365	1061

less than 3·6 per cent. of fat. The largest group in the three years' distribution analysis contained 4·5 per cent. of fat.

The figures for solids-not-fat given in the percentage distribution Tables V and VI, show a much greater uniformity between the morning's and evening's milk, than was the case with the fat. For the whole period the largest group of samples of morning's milk contained 9 per cent. solids-not-fat and of the evening's milk 8·9 per cent.

Daily evening and morning composite tests, averaged for each week of the three years.

Tables VII, VIII and IX show the total weight of milk p.m. and a.m. for each week of the three-year period under discussion.

Table VII. 1928–9. *Showing the number of cows, the weight of milk for each week, ending on the date specified, also the average percentage of fat and solids-not-fat evening and morning.*

Week ending 1928	No. of cows	Weight of milk		Fat percentage		Solids-not-fat percentage	
		p.m. (lb.)	a.m. (lb.)	p.m.	a.m.	p.m.	a.m.
Oct. 7	8	472·5	694·75	4·86	3·58	9·17	9·29
„ 14	8	642·75	939·75	5·14	3·62	9·00	9·19
„ 21	9	631·0	947·25	4·99	3·62	9·06	9·22
„ 28	9	763·5	1089·75	4·78	3·44	8·95	9·17
Nov. 4	8	790·5	1169·5	4·47	3·19	8·84	8·96
„ 11	7	798·25	1115·75	4·33	3·04	8·88	9·06
„ 18	7	748·5	1058·5	4·37	3·06	8·79	9·06
„ 25	7	707·75	1024·25	4·30	3·27	8·89	8·95
Dec. 2	6	664·5	971·25	4·33	3·15	8·83	9·00
„ 9	7	661·25	963·75	4·37	3·11	8·78	8·96
„ 16	6	728·75	1047·75	4·49	3·19	8·89	9·07
„ 23	6	724·5	1062·0	4·34	3·25	8·83	8·96
„ 30	7	751·25	1120·25	4·44	3·33	8·80	8·95
1929							
Jan. 6	7	852·25	1248·0	4·72	3·49	8·84	8·99
„ 13	7	814·0	1219·75	4·64	3·51	8·85	8·96
„ 20	7	826·0	1200·75	4·52	3·29	8·79	8·99
„ 27	7	815·75	1187·75	4·64	3·34	8·78	8·87
Feb. 3	8	848·0	1250·5	4·55	3·20	8·82	8·87
„ 10	8	898·0	1359·25	4·60	3·17	8·86	8·98
„ 17	8	852·25	1312·5	4·66	3·30	8·92	8·98
„ 24	9	845·75	1321·25	4·76	3·37	8·82	8·98
Mar. 3	9	912·0	1464·75	5·11	3·51	8·94	8·99
„ 10	9	935·0	1467·5	5·05	3·37	8·81	8·94
„ 17	9	917·0	1418·75	4·98	3·31	8·91	8·95
„ 24	10	896·25	1393·25	5·16	3·47	8·79	8·92
„ 31	10	1022·0	1564·0	4·84	3·36	8·81	8·97
Apr. 7	10	1031·0	1578·0	4·98	3·21	8·83	8·99
„ 14	10	1018·75	1519·5	4·82	3·20	8·87	8·98
„ 21	10	998·75	1499·75	4·76	3·30	8·82	8·96
„ 28	10	997·5	1483·75	4·52	3·13	8·92	9·00
May 5	9	933·5	1372·25	4·57	3·11	8·89	8·94
„ 12	9	898·75	1308·5	4·74	3·23	8·92	9·02
„ 19	9	927·75	1309·0	4·61	2·95	8·99	9·06
„ 26	9	885·0	1255·75	4·62	2·90	8·91	9·00
June 2	9	831·0	1178·75	4·63	3·02	8·91	9·06
„ 9	9	760·0	1077·75	4·60	2·91	8·79	8·93
„ 16	9	749·0	985·25	4·71	2·86	8·76	8·86
„ 23	9	669·75	951·75	4·61	3·19	8·60	8·77
„ 30	9	594·75	874·5	4·45	3·10	8·74	8·88
July 7	9	544·5	799·5	4·56	3·28	8·79	8·89
„ 14	7	484·75	732·5	4·44	3·25	8·83	8·85
„ 21	7	407·0	673·0	4·66	3·34	8·79	8·92
„ 28	7	431·0	651·25	4·80	3·35	8·77	8·83
Aug. 4	6	396·5	595·75	4·98	3·28	8·71	8·76
„ 11	6	384·75	581·5	4·89	3·51	8·87	8·82
„ 18	6	337·5	571·5	4·91	3·49	8·73	8·78
„ 25	5	331·25	495·25	4·83	3·40	8·82	8·80
Sept. 1	5	312·75	487·5	4·91	3·29	8·70	8·85
„ 8	6	306·5	535·5	4·49	3·47	8·74	8·83
„ 15	7	463·5	773·0	4·60	3·56	8·82	9·02
„ 22	6	510·0	777·5	4·69	3·48	8·76	8·95
„ 29	6	522·5	768·25	4·62	3·33	8·74	8·87

Table VIII. 1929–30.

Week ending 1929	No. of cows	Weight of milk		Fat percentage		Solids-not-fat percentage	
		p.m. (lb.)	a.m. (lb.)	p.m.	a.m.	p.m.	a.m.
Oct. 6	6	489·25	756·25	4·66	3·33	8·76	8·97
„ 13	7	531·0	751·75	5·17	3·52	8·90	9·07
„ 20	7	553·5	798·5	5·00	3·51	8·86	9·10
„ 27	7	556·0	773·25	4·71	3·24	9·01	9·18
Nov. 3	7	488·75	691·0	4·49	3·31	9·00	9·12
„ 10	7	431·5	642·0	4·44	3·31	8·95	9·13
„ 17	7	394·5	622·5	4·22	3·35	8·92	9·05
„ 24	7	458·0	696·5	4·54	3·42	8·91	9·05
Dec. 1	6	530·0	808·0	4·64	3·59	8·98	9·09
„ 8	5	554·75	802·5	4·54	3·32	8·95	8·99
„ 15	5	528·5	792·75	4·53	3·35	8·99	8·96
„ 22	5	522·5	794·25	4·29	3·29	8·93	8·93
„ 29	5	505·25	776·25	4·30	3·19	8·74	8·79
1930							
Jan. 5	5	493·75	751·0	4·30	3·19	8·86	8·83
„ 12	5	481·0	746·0	4·27	3·17	8·84	8·86
„ 19	5	464·0	715·0	4·23	3·30	8·85	8·83
„ 26	5	443·5	683·0	4·40	3·33	8·80	8·80
Feb. 2	5	425·5	669·0	4·23	3·22	8·75	8·78
„ 9	5	388·25	600·0	4·16	3·26	8·79	8·78
„ 16	5	478·75	751·5	4·65	3·56	9·01	8·96
„ 23	5	495·0	744·25	4·55	3·33	9·02	8·99
Mar. 2	6	567·75	828·0	4·84	3·31	9·01	8·96
„ 9	6	625·5	926·25	4·69	3·14	8·95	8·95
„ 16	6	639·75	939·75	4·55	3·04	8·89	8·89
„ 23	8	684·25	1007·0	4·51	3·14	8·77	8·81
„ 30	9	812·0	1232·5	4·35	2·99	8·82	8·88
Apr. 6	9	934·75	1322·5	4·40	2·71	8·89	8·92
„ 13	9	955·75	1312·75	4·33	2·74	8·73	8·88
„ 20	9	981·25	1359·25	4·21	2·78	8·74	8·81
„ 27	9	916·0	1301·5	4·09	2·78	8·74	8·87
May 4	9	932·25	1284·25	4·26	2·75	8·81	8·94
„ 11	9	910·5	1257·5	4·31	2·64	8·85	8·99
„ 18	9	905·75	1186·25	4·32	2·82	8·85	8·97
„ 25	9	872·0	1195·0	4·48	2·77	8·84	8·95
June 1	9	846·5	1098·0	4·48	2·81	8·85	8·91
„ 8	9	764·75	973·25	4·69	2·93	8·68	8·82
„ 15	9	685·0	909·5	4·84	3·11	8·68	8·81
„ 22	9	600·5	801·0	5·05	3·21	8·56	8·75
„ 29	9	569·0	788·25	4·94	3·45	8·61	8·68
July 6	9	513·75	697·25	5·01	3·40	8·46	8·57
„ 13	9	523·75	723·0	4·82	3·46	8·41	8·55
„ 20	8	494·25	680·5	4·80	3·32	8·44	8·55
„ 27	8	451·0	680·5	4·55	3·51	8·45	8·54
Aug. 3	7	465·0	649·75	4·89	3·14	8·42	8·44
„ 10	7	477·5	725·0	4·75	3·25	8·45	8·53
„ 17	7	509·0	758·5	4·50	3·24	8·55	8·67
„ 24	7	504·75	764·5	4·54	3·15	8·65	8·75
„ 31	7	473·5	705·0	4·51	3·30	8·55	8·75
Sept. 7	7	425·25	645·0	4·67	3·30	8·67	8·86
„ 14	7	453·0	648·75	4·66	3·20	8·76	8·78
„ 21	7	419·75	639·5	4·49	3·43	8·84	8·91
„ 28	7	413·0	602·25	4·85	3·37	8·85	8·97

Table IX. 1930–1.

Week ending 1930	No. of cows	Weight of milk p.m. (lb.)	Weight of milk a.m. (lb.)	Fat percentage p.m.	Fat percentage a.m.	Solids-not-fat percentage p.m.	Solids-not-fat percentage a.m.
Oct. 5	7	379·5	557·75	4·84	3·34	8·69	8·81
„ 12	7	429·75	611·0	4·73	3·52	8·88	8·87
„ 19	7	423·25	591·75	4·80	3·51	8·93	8·92
„ 26	8	397·25	565·5	4·81	3·56	8·90	8·92
Nov. 2	8	427·0	647·5	4·71	3·65	8·96	9·05
„ 9	9	603·25	877·5	5·06	3·76	9·01	9·10
„ 16	9	573·5	828·75	5·05	3·65	8·92	9·14
„ 23	9	513·75	787·25	4·90	3·58	8·89	8·99
„ 30	9	508·5	774·75	4·56	3·46	8·96	8·97
Dec. 7	8	460·75	709·25	4·74	3·49	8·71	8·93
„ 14	8	447·25	679·5	4·68	3·55	8·89	9·05
„ 21	9	487·25	740·25	4·42	3·56	8·89	9·04
„ 28	9	459·25	754·25	4·36	3·40	8·91	8·99
1931							
Jan. 4	8	483·75	772·75	4·31	3·33	8·89	8·98
„ 11	7	493·5	748·0	4·56	3·28	8·91	9·06
„ 18	7	484·5	747·0	4·37	3·20	8·89	8·95
„ 25	7	467·0	737·0	4·25	3·14	8·91	8·93
Feb. 1	6	443·0	687·0	4·23	3·01	8·93	8·93
„ 8	6	413·25	650·75	4·19	3·11	8·98	8·93
„ 15	5	404·25	607·75	4·22	3·16	8·94	8·93
„ 22	5	385·5	597·5	4·26	3·11	8·91	8·95
Mar. 1	5	384·5	600·5	4·19	3·07	8·91	8·94
„ 8	6	461·25	695·75	4·32	3·36	8·92	8·92
„ 15	6	484·5	723·0	4·38	3·07	8·94	9·02
„ 22	7	577·0	884·25	4·30	3·20	8·84	8·95
„ 29	8	596·0	903·75	4·34	3·11	8·87	8·96
Apr. 5	8	688·0	1040·5	4·62	3·06	8·91	9·03
„ 12	8	702·75	1081·25	4·44	2·93	8·83	9·01
„ 19	8	709·0	1073·5	4·39	3·01	8·82	8·90
„ 26	8	711·5	1063·25	4·35	3·05	8·83	8·92
May 3	8	765·5	1117·25	4·27	2·97	8·88	8·98
„ 10	8	772·0	1154·5	4·15	2·79	8·79	8·95
„ 17	8	767·0	1184·75	4·10	2·88	8·88	8·99
„ 24	8	746·5	1127·0	4·12	2·82	8·83	9·01
„ 31	9	764·5	1164·5	4·25	3·04	8·88	9·05
June 7	10	842·75	1321·75	4·42	3·13	8·85	9·07
„ 14	9	908·0	1381·25	4·28	3·03	8·90	9·04
„ 21	9	913·75	1352·5	4·27	2·89	8·81	8·98
„ 28	9	901·5	1243·25	4·43	2·93	8·75	8·94
July 5	9	922·5	1279·25	4·28	3·01	8·78	9·06
„ 12	9	899·5	1237·25	4·31	2·96	8·70	8·93
„ 19	9	845·25	1214·25	4·31	2·99	8·78	8·89
„ 26	9	834·75	1163·0	4·30	3·10	8·72	8·93
Aug. 2	9	801·25	1098·75	4·29	3·11	8·79	8·95
„ 9	9	721·5	1060·75	4·48	3·08	8·73	8·93
„ 16	8	681·75	980·5	4·68	3·20	8·78	9·02
„ 23	8	656·25	919·25	4·65	3·33	8·83	9·04
„ 30	8	626·25	874·25	4·66	3·29	8·80	9·04
Sept. 6	8	567·5	816·0	4·63	3·47	8·87	9·03
„ 13	8	575·0	844·5	4·64	3·47	8·97	9·06
„ 20	8	613·25	865·75	4·69	3·33	8·89	9·06
„ 27	8	565·5	806·5	4·62	3·21	8·89	9·06

The number of cows in milk during each week is shown in the second column.

The percentages of fat p.m. and a.m. and of the solids-not-fat p.m. and a.m. are shown in columns 5–8 and are the calculated averages for each week.

Discussion of Tables VII–IX.

The number of cows shown in column 2, which were in milk at the dates shown in column 1, is subject to variation due to cows drying off and newly calved cows coming in milk.

The average percentage of fat in the morning's milk, column 6, fell below 3 per cent. during 4 weeks in 1929, 11 weeks in 1930 and 9 weeks in 1931 (see Tables VII–IX). This low result was always found in the spring months. There was a general tendency for the percentages of fat to rise, as the quantity of milk fell, due to advancing lactation.

The solids-not-fat, columns 7 and 8, were fairly high and regular. One exception to this may be noted on reference to Table VIII, where low results are seen in the averages for the weeks from July 6 to August 10. This was the period when changes in feeding were made owing to an attack of "husk."

Weekly analyses of a composite sample of morning milk.

The analyses of these composite samples, prepared in the same way as the daily samples (see p. 54), were made by the following methods. All the determinations were made in duplicate and standard apparatus was used.

Acidity. The acidity was determined by titrating 10 ml. of milk with *N*/9 NaOH, using phenolphthalein as an indicator. Ten ml. of milk to which 1·5–2·0 ml. of *N*/9 NaOH were added were used as a colour control. The results are expressed as per cent. lactic acid, and are shown in Tables X, XI and XII. The greatest difference between duplicate determinations in any year was 0·004. The results throughout the whole three-year period were very uniform, the highest value being 0·18 per cent. and the lowest 0·14 per cent.

The average value for each of the three years was 0·16, 0·16 and 0·15 per cent. respectively. There were indications of a slightly lower acidity when the cows were on pasture (see Tables XI and XII).

Formol titration. Ten ml. of commercial formalin (40 per cent.) diluted with its own volume of water, and neutralised to phenolphthalein with *N*/9 NaOH, was added to the neutralised milk used in the acidity determination, and the titrations with *N*/9 NaOH continued until the pink colour reappeared. The number of ml. of *N*/9 NaOH necessary to restore the colour to the milk are shown in Tables X, XI and XII as "formol titration." The greatest difference between duplicate results was 0·06 (1928–9) and 0·04 c.c. (1929–30 and 1930–1). The average for 1928–9 was 1·52 with the highest and lowest values of 1·63 and 1·42; for 1929–30 the average was 1·42 with the highest and lowest values of 1·59 and 1·20, and for 1930–1 the average value was 1·40 and the highest and lowest values were 1·51 and 1·31 respectively.

The results are very uniform throughout, but the higher results occur in the winter months. By reference to Tables X, XI and XII it is seen that during the greater part of the experimental period there is some positive correlation with the titratable acidity.

Table X.

Date 1928	Titratable acidity (*N*/9 NaOH)	Formol titration (*N*/9 NaOH)	Rennet test min.	Rennet test sec.	Total nitrogen %	Casein nitrogen %
Oct. 17	—	—	—		0·534	0·409
„ 23	0·17	—	—		0·517	0·396
„ 30	0·16	—	—		0·500	0·387
Nov. 6	0·16	1·50	—		0·502	0·391
„ 13	0·16	1·44	—		0·504	0·388
„ 20	0·16	1·57	—		0·493	0·382
„ 27	0·17	1·49	—		—	—
Dec. 4	0·16	1·42	—		0·504	0·382
„ 11	0·17	1·63	—		0·535	0·405
„ 18	0·17	1·50	—		0·500	0·400
1929						
Jan. 1	0·16	1·54	3	22	0·545	0·425
„ 8	0·16	1·56	3	6	0·520	0·410
„ 15	0·17	1·52	3	9	0·506	0·397
„ 22	0·16	1·51	3	6	0·520	0·390
„ 29	0·16	1·51	3	12	0·531	0·406
Feb. 5	0·17	1·56	2	51	0·521	0·408
„ 12	0·17	1·57	3	5	0·509	0·398
„ 19	0·17	1·62	2	49	0·515	—
„ 26	0·17	1·54	2	29	0·530	0·415
Mar. 5	0·17	1·54	2	33	0·520	0·400
„ 12	0·17	1·57	2	31	0·509	0·398
„ 19	0·16	1·50	2	48	0·514	0·388
„ 26	0·17	1·54	2	18	0·525	0·410
Apr. 11	0·16	1·56	2	19	0·525	0·406
„ 18	0·17	1·51	2	7	0·530	0·405
„ 23	0·16	1·53	2	26	0·518	0·404
„ 30	0·16	1·49	2	20	0·530	0·400
May 7	0·16	1·52	2	15	0·550	0·420
„ 14	0·17	1·51	2	32	0·540	0·420
„ 21	0·16	1·49	2	42	0·545	0·400
„ 28	0·16	1·51	2	39	0·551	0·392
June 4	0·16	1·49	2	38	0·531	0·388
„ 11	0·15	1·57	2	37	0·504	0·410
„ 18	0·16	1·49	2	27	0·517	0·400
„ 25	0·17	1·50	2	21	0·526	0·420
July 2	0·16	1·49	2	29	0·520	0·417
„ 9	0·15	1·47	2	27	0·527	0·420
„ 16	0·16	1·51	2	29	0·533	0·421
„ 23	0·15	1·49	2	31	0·522	0·412
„ 30	0·15	1·42	2	2	0·499	0·395
Aug. 6	0·16	1·49	2	16	0·517	0·417
„ 13	0·14	1·48	2	33	0·513	0·397
„ 20	0·15	1·49	2	32	0·495	0·399
„ 27	0·17	1·49	1	51	0·532	0·407
Sept. 3	0·16	1·52	1	57	0·526	0·412
„ 10	0·16	1·51	1	55	0·546	0·418
„ 17	0·16	1·50	2	5	0·530	0·412
„ 24	0·16	1·51	2	14	0·520	0·390

Table XI

Date 1929	Titratable acidity (*N*/9 NaOH)	Formol titration (*N*/9 NaOH)	Rennet test min.	Rennet test sec.	Total nitrogen %	Casein nitrogen %
Oct. 1	0·15	1·48	2	22	0·506	0·403
„ 8	0·17	1·50	2	11	0·533	0·398
„ 15	0·16	1·49	2	15	0·546	0·399
„ 22	0·17	1·51	2	17	0·546	0·430
„ 29	0·16	1·51	2	15	0·554	0·435
Nov. 5	0·16	1·50	2	35	0·580	0·440
„ 12	0·16	1·51	2	44	0·545	0·418
„ 19	0·17	1·47	2	23	0·522	0·370
„ 26	0·18	1·63	1	59	0·538	0·420
Dec. 3	0·18	1·53	2	31	0·505	0·401
„ 10	0·18	1·53	2	29	0·515	0·392
„ 17	0·18	1·52	2	32	0·512	0·394
„ 31	0·18	1·50	3	9	0·523	0·406
1930						
Jan. 7	0·17	1·51	3	4	0·519	0·405
„ 14	0·17	1·59	3	18	0·519	0·397
„ 21	0·17	1·49	4	1	0·518	0·351
„ 28	0·16	1·49	3	58	0·525	0·400
Feb. 4	0·17	1·53	3	58	0·519	0·405
„ 11	0·18	1·53	3	32	0·550	0·428
„ 18	0·17	1·53	3	20	0·542	0·424
„ 25	0·17	1·49	3	29	0·543	0·420
Mar. 4	0·17	1·49	2	22	0·530	0·416
„ 11	0·16	1·44	2	50	0·527	0·410
„ 18	0·16	1·51	2	47	0·525	0·398
„ 25	0·16	1·46	2	15	0·537	0·403
Apr. 1	0·16	1·48	2	22	0·525	0·401
„ 8	0·16	1·41	2	6	0·529	0·396
„ 15	0·15	1·44	2	23	0·523	0·386
„ 22	0·15	1·43	2	25	0·512	0·404
„ 29	0·15	1·47	2	16	0·543	0·419
May 6	0·15	1·43	2	18	0·539	0·419
„ 13	0·15	1·41	2	22	0·529	0·400
„ 20	0·15	1·37	2	26	0·540	0·408
„ 27	0·15	1·33	2	39	0·524	0·404
June 3	0·15	1·31	2	40	0·527	0·395
„ 10	0·15	1·37	—		—	—
„ 17	0·15	1·30	2	34	0·496	0·374
„ 24	0·15	1·37	2	31	0·506	0·391
July 1	0·15	1·34	2	32	0·501	0·374
„ 8	0·14	1·29	1	57	0·499	0·384
„ 15	0·15	1·30	2	11	0·507	0·380
„ 22	0·14	1·20	1	59	0·518	0·397
„ 29	0·15	1·29	2	9	0·508	0·370
Aug. 5	0·15	1·24	2	8	0·525	0·391
„ 12	0·15	1·31	2	6	0·533	0·393
„ 19	0·15	1·30	2	7	0·528	0·397
„ 26	0·15	1·29	2	9	0·537	0·393
Sept. 2	0·15	1·31	2	10	0·546	0·385
„ 9	0·15	1·34	2	6	0·527	0·374
„ 16	0·15	1·29	2	9	0·540	0·408
„ 23	0·15	1·39	2	8	0·563	0·424
„ 30	0·15	1·39	2	6	0·586	0·417

Table XII.

Date 1930	Titratable acidity (*N*/9 NaOH)	Formol titration (*N*/9 NaOH)	Rennet test min.	Rennet test sec.	Total nitrogen %	Casein nitrogen %
Oct. 7	0·15	1·32	2	9	0·572	0·420
„ 14	0·15	1·32	2	12	0·570	0·418
„ 21	0·15	1·31	2	7	0·577	0·422
„ 28	0·15	1·37	1	54	0·560	0·422
Nov. 4	0·15	1·39	2	4	0·576	0·437
„ 11	0·15	1·44	2	7	0·558	0·424
„ 18	0·15	1·42	2	14	0·558	0·422
„ 25	0·15	1·47	2	29	0·541	0·410
Dec. 2	0·14	1·45	2	36	0·531	0·430
„ 9	0·15	1·45	2	28	0·545	0·412
„ 16	0·15	1·46	2	37	0·573	0·440
„ 23	0·16	1·42	2	36	0·553	0·419
1931						
Jan. 6	0·16	1·49	2	40	0·553	0·419
„ 13	0·16	1·49	2	53	0·536	0·402
„ 20	0·17	1·51	2	57	0·505	0·399
„ 27	0·16	1·51	3	2	0·521	0·413
Feb. 3	0·16	1·40	3	23	0·526	0·396
„ 10	0·16	1·41	3	25	0·518	0·398
„ 17	0·15	1·39	3	43	0·544	0·424
„ 24	0·15	1·38	3	57	0·526	0·407
Mar. 3	0·15	1·40	3	20	0·539	0·417
„ 10	0·15	1·33	3	8	0·546	0·429
„ 17	0·16	1·41	2	44	0·554	0·421
„ 24	0·16	1·39	2	44	0·540	0·415
„ 31	0·16	1·40	2	38	0·545	0·418
Apr. 7	0·16	1·39	2	14	0·540	0·413
„ 14	0·16	1·41	2	9	0·516	0·402
„ 21	0·15	1·39	2	12	0·505	0·394
„ 28	0·15	1·39	2	28	0·518	0·406
May 5	0·15	1·37	2	29	0·507	0·407
„ 12	0·15	1·40	2	32	0·539	0·405
„ 19	0·15	1·42	2	47	0·563	0·419
„ 26	0·15	1·39	2	53	0·535	0·416
June 2	0·15	1·41	2	47	0·575	0·415
„ 9	0·15	1·37	2	39	0·563	0·402
„ 16	0·15	1·39	2	22	0·542	0·401
„ 23	0·15	1·41	2	22	0·537	0·407
„ 30	0·15	1·41	2	40	0·568	0·436
July 7	0·15	1·40	2	47	0·534	0·419
„ 14	0·15	1·31	2	28	0·523	0·410
„ 21	0·15	1·34	2	22	0·539	0·408
„ 28	0·15	1·41	2	26	0·525	0·415
Aug. 4	0·15	1·39	2	15	0·530	0·403
„ 11	0·15	1·36	2	4	0·544	0·419
„ 18	0·15	1·43	2	22	0·549	0·422
„ 25	0·15	1·35	2	21	0·541	0·427
Sept. 1	0·15	1·38	2	7	0·553	0·423
„ 8	0·15	1·44	1	53	0·563	0·409
„ 15	0·15	1·39	1	58	0·562	0·428
„ 22	0·15	1·34	1	52	0·552	0·424
„ 29	0·16	1·46	1	53	0·563	0·416

Rennet test. The method used for determining the time of coagulation with rennet was that already described by Mattick and Hallett (1). Two hundred and fifty ml. of milk and 3 ml. of a 1:3 solution of rennet were used. The error between duplicate determinations was never greater than 5 sec., the highest and lowest value obtained respectively for the three years were 3 min. 22 sec. and 1 min. 51 sec. (1928–9), 4 min. 1 sec. and 1 min. 57 sec. (1929–30), and 3 min. 57 sec. and 1 min. 52 sec. for 1930–1.

Very definite slowing down of the rennet test occurs during the winter months, particularly in January and February, when the time taken to coagulate the milk may be almost double the time taken in summer.

This marked seasonal variation in the time of coagulation by rennet probably underlies some of the difficulties experienced in winter cheese-making, and merits further investigation.

Total nitrogen. Total nitrogen was determined on 5 ml. milk by the Kjeldahl method. The greatest differences between duplicates were 0·017, 0·018 and 0·014 respectively for the three years. The average value for 1928 was 0·521 per cent. with the highest and lowest values of 0·546 and 0·493 per cent. In 1929–30 the average was 0·529 per cent. with highest and lowest values of 0·586 and 0·505 per cent., and for 1930–1 the average was 0·544 per cent., the highest value was 0·577 per cent., and the lowest 0·505 per cent.

The high values shown in May, 1929, may possibly be associated with grazing of young grass, which has been shown by Woodman (2) to be rich in nitrogen.

Relatively high values were found at other periods of the year, notably in the autumn of 1930, but the rise in the spring is repeated in subsequent years.

Casein nitrogen. Ten ml. of milk were measured with a standard pipette into a 100 ml. beaker. Fifty ml. of distilled water were added at 35–40° C. To this, 1·5 ml. of a 10 per cent. acetic acid solution was added and after a few minutes was stirred gently and then allowed to stand for at least 30 minutes. The precipitate was filtered off, and washed with distilled water until free from acid. The filter paper and precipitate were transferred to a Kjeldahl flask. Any precipitate remaining in the beaker was dissolved in H_2SO_4 and added to the precipitate in the Kjeldahl flask, and the nitrogen determination carried out in the usual way.

The greatest differences between duplicates was 0·02 in the years 1928–9, and 1929–30, and was only 0·008 throughout 1930–1. The average value for 1928–9 was 0·403 per cent. with greatest and least values of 0·421 and 0·382 per cent.; for 1929–30 the average was 0·401 per cent. with greatest and least values of 0·440 and 0·351, while for 1930–1 the average was 0·415 with greatest and least values of 0·437 and 0·394 per cent.

The results throughout the period of the experiment run roughly parallel to those of the total nitrogen.

Turning to Tables XIII, XIV and XV, the yield of milk on the dates

Table XIII.

Date 1928	Yield in lb.	Fat %	Solids-not-fat %	Ash %	Calcium %	Phosphorus %
Oct. 17	132¾	3·45	9·25	—	0·136	0·113
„ 23	153½	3·80	9·12	0·79	0·136	0·109
„ 30	162¼	3·40	8·97	0·80	0·134	0·098
Nov. 6	158¾	3·15	9·02	0·77	0·128	0·112
„ 13	153¾	2·55	9·12	0·78	0·129	0·114
„ 20	143¾	3·30	8·99	0·78	0·130	0·110
„ 27	139¼	3·20	9·00	0·71	0·127	0·111
Dec. 4	130¾	3·00	8·88	0·78	0·127	0·110
„ 11	152¾	3·25	9·03	0·76	0·126	0·114
„ 18	147¼	3·30	8·87	0·76	0·127	0·113
1929						
Jan. 1	179¾	3·50	8·99	0·77	0·128	0·115
„ 8	177½	3·55	8·99	0·73	0·128	0·114
„ 15	174½	3·35	8·89	0·75	0·126	0·114
„ 22	173¼	3·30	9·01	0·77	0·125	0·117
„ 29	166¾	3·30	8·97	0·75	0·127	0·116
Feb. 5	193½	3·05	8·97	0·74	0·123	0·117
„ 12	188½	3·20	8·86	0·70	0·124	0·114
„ 19	183¾	3·25	8·99	0·71	0·126	0·116
„ 26	210¾	3·50	8·99	0·87	0·125	0·117
Mar. 5	206¼	3·35	8·88	0·79	0·125	0·113
„ 12	194¾	3·35	8·94	0·78	0·126	0·115
„ 19	186¼	3·40	8·90	0·79	0·126	0·113
„ 26	221	3·20	8·97	0·87	0·128	0·118
Apr. 11	216½	3·10	8·98	0·84	0·125	0·115
„ 18	220¼	3·35	9·00	0·81	0·130	0·113
„ 23	211½	3·10	9·05	0·84	0·131	0·116
„ 30	199½	3·05	8·89	0·85	0·129	0·112
May 7	184½	3·25	9·01	0·77	0·129	0·109
„ 14	187½	3·00	9·01	0·75	0·127	0·109
„ 21	185	2·80	8·91	0·76	0·125	0·109
„ 28	167½	3·00	9·03	0·73	0·127	0·109
June 4	155	2·80	9·08	0·80	0·127	0·104
„ 11	140½	2·80	8·84	0·77	0·122	0·105
„ 18	131¼	3·05	8·76	0·82	0·124	0·106
„ 25	126¾	2·90	8·87	0·77	0·124	0·103
July 2	111¼	3·00	8·93	0·79	0·122	0·103
„ 9	104¾	3·50	8·81	0·83	0·126	0·099
„ 16	93¾	3·40	8·82	0·81	0·127	0·099
„ 23	97¾	3·45	8·86	0·80	0·123	0·099
„ 30	83¼	3·30	8·76	0·75	0·126	0·096
Aug. 6	88¼	3·40	8·89	0·79	0·124	0·098
„ 13	85	3·70	8·71	0·75	0·127	0·097
„ 20	70¼	3·50	8·74	0·78	0·126	0·097
„ 27	69¾	3·10	8·90	0·80	0·131	0·099
Sept. 3	62	3·20	8·91	0·82	0·130	0·095
„ 10	111	3·60	9·01	0·80	0·134	0·104
„ 17	105	3·40	9·00	0·80	0·132	0·103
„ 24	111¼	3·30	8·78	0·78	0·130	0·103

Table XIV.

Date 1929	Yield in lb.	Fat %	Solids-not-fat %	Ash %	Calcium %	Phosphorus %	Lactose %
Oct. 1	111¼	3·30	8·93	0·79	0·136	0·111	—
„ 8	99½	3·50	9·11	0·78	0·132	0·106	—
„ 15	109¾	3·50	9·10	0·79	0·136	0·113	—
„ 22	112¼	3·20	9·08	0·81	0·133	0·103	—
„ 29	104½	3·10	9·14	0·85	0·137	0·113	—
Nov. 5	95	3·40	9·11	0·85	0·140	0·114	—
„ 12	85¾	3·50	9·12	0·0	0·135	0·110	—
„ 19	97½	3·60	8·96	—	0·132	0·112	—
„ 26	116¼	3·90	9·16	0·85	0·134	0·118	—
Dec. 3	111¾	3·30	9·02	0·77	0·128	0·117	—
„ 10	111¾	3·60	8·89	0·75	0·124	0·113	—
„ 17	112½	3·55	8·86	0·72	0·124	0·111	—
„ 31	104	3·10	8·86	0·78	0·122	0·114	—
1930							
Jan. 7	107¼	3·15	8·82	0·75	0·121	0·116	—
„ 14	99	3·20	8·79	0·75	0·120	0·114	—
„ 21	97¾	3·00	8·94	0·76	0·120	0·118	—
„ 28	92¾	3·40	8·74	0·77	0·118	0·116	—
Feb. 4	84¾	3·05	8·69	0·75	0·116	0·121	—
„ 11	110¼	3·65	8·98	0·78	0·123	0·123	—
„ 18	103	3·45	9·04	0·77	0·125	0·115	—
„ 25	106	3·30	8·89	0·77	0·122	0·114	—
Mar. 4	130¼	3·20	8·89	0·76	—	0·111	—
„ 11	133½	3·00	8·95	0·77	0·121	0·113	4·78
„ 18	140	3·15	8·89	0·76	0·120	0·111	4·80
„ 25	174¾	3·05	8·82	0·77	0·122	0·112	4·85
Apr. 1	184½	2·70	8·89	0·77	0·123	0·109	4·87
„ 8	185¾	2·80	8·85	0·75	0·123	0·105	4·89
„ 15	210¼	2·65	8·70	0·76	0·119	0·106	4·93
„ 22	182½	2·80	8·92	0·76	0·120	0·107	4·90
„ 29	181¾	2·85	8·94	0·75	0·131	0·105	4·91
May 6	194¼	2·80	8·92	0·76	—	0·108	4·87
„ 13	164½	2·65	8·96	0·75	0·119	0·106	4·77
„ 20	169¼	2·75	8·91	0·75	0·120	0·108	4·85
„ 27	163½	2·80	8·80	0·77	0·120	0·105	4·89
June 3	144	2·90	8·81	0·75	0·120	—	4·88
„ 10	139¾	3·15	8·84	0·76	0·122	0·103	—
„ 17	116¾	3·40	8·77	0·74	0·121	0·099	4·73
„ 24	116¼	3·70	8·77	0·76	0·123	0·099	4·70
July 1	107	3·40	8·57	0·75	0·118	0·095	4·55
„ 8	110¼	3·50	8·57	0·77	0·119	0·099	4·58
„ 15	98	3·40	8·61	0·76	0·115	0·095	4·61
„ 22	98¼	4·00	8·48	0·74	0·118	0·095	4·62
„ 29	100¼	3·30	8·36	0·73	0·115	0·092	4·56
Aug. 5	102¼	3·10	8·42	0·75	0·125	0·096	4·44
„ 12	104½	3·25	8·67	0·76	0·122	0·098	4·73
„ 19	105¼	3·00	8·59	0·77	0·117	0·097	4·57
„ 26	100	3·30	8·86	0·76	0·120	0·095	4·55
Sept. 2	94	3·40	8·83	0·75	0·124	0·099	4·69
„ 9	96¾	3·30	8·73	0·78	0·132	0·105	4·62
„ 16	87¾	3·20	8·84	0·77	0·133	0·102	—
„ 23	87¼	3·40	8·92	0·79	0·132	0·103	—
„ 30	78½	3·50	8·98	0·79	0·133	0·105	4·53

Table XV.

Date 1930	Yield in lb.	Fat %	Solids-not-fat %	Ash %	Calcium %	Phosphorus %	Lactose %
Oct. 7	92½	3·50	8·83	0·78	0·138	0·105	4·45
„ 14	86½	3·55	8·95	0·78	0·134	0·107	4·53
„ 21	85	3·50	8·94	0·79	0·138	0·105	4·52
„ 28	74½	3·50	8·92	0·78	0·133	0·103	4·49
Nov. 4	124½	3·95	9·09	0·80	0·141	0·109	4·70
„ 11	122½	3·60	9·15	—	—	—	—
„ 18	111½	3·80	9·04	0·78	0·137	0·104	4·72
„ 25	112½	3·40	8·92	0·79	0·132	0·107	—
Dec. 2	104¼	3·25	8·99	0·79	0·129	0·103	4·69
„ 9	92	3·50	8·83	0·77	0·134	0·105	4·70
„ 16	103¾	3·50	9·06	0·79	0·137	0·108	4·70
„ 23	106¾	3·50	9·03	0·77	0·135	0·104	4·77
1931							
Jan. 6	106¼	3·20	8·99	0·81	0·126	0·105	4·85
„ 13	107½	3·10	8·89	0·81	0·125	0·108	4·85
„ 20	106¾	3·10	8·91	0·77	0·126	0·108	4·85
„ 27	98½	3·00	8·89	0·78	0·137	0·106	4·95
Feb. 3	93¼	3·15	8·82	0·75	0·138	0·102	4·90
„ 10	85¼	3·30	8·83	0·76	0·136	0·102	4·77
„ 17	83¾	3·20	9·03	0·77	0·138	0·105	4·84
„ 24	85¾	3·00	8·91	0·79	0·129	0·105	4·74
Mar. 3	100½	3·40	8·85	0·80	0·130	0·104	4·65
„ 10	102	3·20	9·05	0·81	0·132	0·103	4·86
„ 17	131½	3·20	9·02	0·78	0·131	0·105	4·83
„ 24	126½	3·20	8·90	0·75	0·127	0·107	4·97
„ 31	143¼	3·10	9·03	0·80	0·128	0·107	4·87
Apr. 7	151½	3·00	8·97	0·78	0·131	0·103	4·90
„ 14	152¾	3·00	8·89	0·78	0·124	0·099	4·87
„ 21	141¼	3·00	8·90	0·76	0·128	0·096	4·89
„ 28	157¾	3·20	8·91	0·79	0·132	0·101	4·98
May 5	157½	2·75	8·97	0·73	0·131	0·103	4·81
„ 12	171½	2·90	9·03	0·75	0·129	0·103	4·77
„ 19	165	2·85	8·99	0·74	0·127	0·103	4·82
„ 26	157	2·70	9·07	0·76	0·126	0·100	4·82
June 2	171	3·05	8·96	0·77	0·126	0·105	—
„ 9	195½	2·80	9·10	0·77	0·130	0·106	4·75
„ 16	191¾	2·85	9·00	0·76	0·128	0·104	4·82
„ 23	178½	2·90	8·93	0·76	0·128	0·103	4·86
„ 30	174¾	3·05	9·05	0·78	0·127	0·104	4·83
July 7	181½	3·10	8·85	0·78	0·129	0·103	4·82
„ 14	171½	3·10	8·81	0·77	0·128	0·103	4·79
„ 21	167¾	2·90	8·90	0·78	0·126	0·100	4·82
„ 28	157	3·20	8·90	0·77	0·127	0·101	4·77
Aug. 4	155½	3·10	9·02	0·76	0·130	0·100	4·80
„ 11	142½	3·10	8·93	0·77	0·128	0·103	4·77
„ 18	131½	3·20	8·98	0·77	0·130	0·101	4·78
„ 25	125¾	3·60	9·04	0·77	0·133	0·099	4·76
Sept. 1	118½	3·50	9·02	0·78	0·132	0·102	4·72
„ 8	116¾	3·10	9·12	0·77	0·134	0·103	4·70
„ 15	125½	3·45	9·18	0·79	0·135	0·102	4·63
„ 22	121¾	3·25	9·06	0·78	0·135	0·105	4·72
„ 29	97¾	3·30	8·99	0·77	0·134	0·106	4·75

specified in column 1 is given in lb. in column 2. The percentages of fat and solids-not-fat in this milk are given in columns 3 and 4. The methods used for the determination of these constituents are given on p. 54 and were the same as those used for all the daily analyses.

The solids-not-fat were calculated by subtracting the values for fat from the mean of the total solid determinations. Duplicate results of total solids agreed very closely, the extreme differences in 148 determinations were as follows: six differed by 0·06, two by 0·07, one by 0·08 and one by 0·12.

Ash. The ash was determined in 25 ml. of milk which was weighed into a silica or porcelain crucible, evaporated to dryness, and ashed at a temperature not exceeding a dull red heat in an electric furnace. When the ash was well charred it was moistened with water, re-evaporated, returned to the furnace and the ashing continued until a white ash of constant weight was obtained.

The average value for the year 1928–9 was 0·78 per cent., the highest value (which occurred only twice) was 0·87 and the lowest 0·70 per cent. The greatest difference between duplicates was 0·06. Throughout this period the ashing was carried out on an Argand burner, and was therefore not very satisfactory. No other means were at that time available. No periodical changes in the value for the ash were observed.

During the second period, October, 1929 to September, 1930, the average value was very close to that in the previous year, namely 0·77 per cent.; the differences between the extreme values was slightly less, the percentages being 0·85 and 0·72. The greatest difference between duplicates was 0·04, which occurred during September and October, 1929. The difference was not above 0·02 per cent. between October 22, 1929 and September 9, 1930. After November 12, 1929, the ashing process was carried out in an electric furnace. The higher values occurring in October and November, 1929, and again in September, 1930, appear to show a positive correlation with the calcium values for those periods. The average value for the third year was again 0·78 per cent., and highest and lowest percentages being 0·81 and 0·73 respectively. The greatest difference between duplicates was 0·03. No periodical change in the value for the ash was observed.

Calcium. The calcium was estimated in the ash by the McCrudden method (3, 4).

During 1928–9, the highest and lowest percentages of calcium found were 0·136 and 0·122, with an average value of 0·127 and an experimental error between duplicates of 0·006. The values are very uniform during the period November, 1928 to August, 1929, but were higher at the beginning and end of the experimental period, during October, 1928 and September, 1929. It is suggested that these higher values may be associated with the feeding of cabbage in 1928 and kale in 1929.

The highest percentage of calcium found during the second experimental period was 0·140 and the lowest 0·115, the average being 0·124, the greatest difference between duplicates was 0·004. High values occurred at the be-

ginning and end of the period as in 1928–9. There appears to be a positive correlation with the solids-not-fat during these months, and a possible association with the feeding of cabbage during October to November, 1929, and lucerne in September, 1929.

The average value for 1930–1 was slightly higher than for either of the preceding ones, being 0·131, and the greatest and least values throughout the period were 0·141 and 0·124. The greatest difference between duplicates was 0·004. The values throughout the whole of this experimental period tended to be rather higher than in previous years, but although the differences are not so marked, the higher values appear during the same months as previously, *i.e.* at the beginning and end of the period.

Phosphorus. Phosphate was estimated on the filtrate from the calcium determination by a slightly modified Neumann's method (5).

After evaporation to dryness on a water bath, 5 ml. of strong HNO_3 were added and evaporated. This was once repeated. The residue was taken up by boiling with 50 ml. of distilled water and the phosphate precipitated by ammonium molybdate in nitric acid (140 ml. of a solution containing 50 g. molybdic acid in 100 ml. water and 100 ml. of strong NH_4OH, and 720 ml. of HNO_3 – S.G. 1·2). The precipitation was carried out at about 60° C., the solution stirred rapidly and allowed to stand for at least 24 hours. After filtering off, washing with 0·5 per cent. KNO_3 solution until free from HNO_3 (tested by methyl orange), the precipitate was dissolved in excess of $N/2$ NaOH and the excess titrated with $N/2$ HNO_3, using phenolphthalein as indicator.

The average value for phosphorus during the period 1928–9 was 0·108 per cent., the highest and lowest amounts being 0·118 and 0·095, the greatest difference between duplicates was 0·004. The values remained practically unchanged from October, 1928 to May, 1929, when lower values were obtained until the end of September. The lowest values, from July 16 to September 3, correspond to the period of lowest yield, due to drying off. At this time the cows were feeding on aftermath with additional silage. On September 8, marrow-stem kale was fed, and there was a slight rise in the percentage of phosphorus found, which although very small is perhaps significant.

In 1929–30 the highest value for phosphorus was 0·123 and the lowest 0·092 per cent., the average being 0·107 per cent. Very uniform quantities were found until the beginning of April, when a decrease comparable with that in 1928–9 was shown. These low values persist until the end of the experimental period, being particularly low from the middle of June until early September. These lower values again appear to be associated with the grazing period and the feeding of vetches, lucerne and hay, but not directly with lowered milk yield.

During the third experimental period, October, 1930 to September, 1931, the average amount of phosphorus found was 0·104 per cent., the highest quantity being 0·109 and the lowest 0·096 per cent. The greatest difference

between duplicate determinations was 0·005. During these twelve months the amount of phosphorus is very uniform, no periods with higher or lower values being observable. Ample pasture was available and no additional feeds were given during the summer months until September when a little lucerne was given.

Lactose. The lactose was not recorded till February, 1930, after which time determinations were carried out by the American Official Optical method (6). The results have also been repeated by the gravimetric method of Munson and Walker (13), in which the precipitate is weighed as cuprous oxide.

The figures given in Tables XIV and XV are the average between two results obtained by the optical method. The gravimetric results were also determined in duplicate, and since June 23, 1931 have agreed very closely with the optical method, the maximum difference between the two methods, which occurred on one occasion only, September 8, 1931, was 0·12 per cent., the optical result being higher than the gravimetric result.

The freezing-point.

The freezing-point was determined every week from March 19, 1929. The method of Andrew (7) was employed. Three solid stem thermometers were used. No Raoult correction was made, so that the apparent freezing-point only is recorded.

In spite of these variations all but three results fell between − 0·555 and − 0·540° C., on the other three occasions freezing-points of − 0·538° C. were recorded.

An N.P.L. standard solid stem thermometer is now available, and it will be possible to make more accurate determinations. It is however clear that the freezing-point of this type milk is subject to very slight variation. The variations on either side of the mean (− 0·547) being little greater than the experimental error.

Total solids by calculation and by estimation.

The total solids were calculated from the specific gravity determinations, and the fat determinations by means of the Droop Richmond milk scale. The formulae being

$$\text{Total solids} = \frac{G}{4} + 1{\cdot}2F + 0{\cdot}14,$$

where $G = 1000$ (specific gravity) $- 1000$ (*i.e.* lactometer degrees of the milk), and $F =$ fat percentage of the milk.

This method is in common use for the approximate estimation of the total solids.

The chart, Fig. 1, shows the mean monthly errors for the period of three years. Two outstanding facts are obvious from this chart. The first is that the monthly average of the morning milks invariably gave a lower calculated

total solids content than the corresponding evening milks (age of milk on taking the S.G. was about 4 hours and 14 hours respectively), and the second is that in winter the calculated total solids tended to be higher than in summer (8).

This phenomenon was further investigated, and it seems to be due to at least two causes.

The first is brought about by changes which take place in the physical condition of the fat globules, and is associated with the variable rise in the specific gravity which takes place in milk on standing for hours at different temperatures.

The second is due to changes in the specific gravity of the solids-not-fat. This change is very marked when cows are in an advanced stage of lactation. The whole subject will be investigated further.

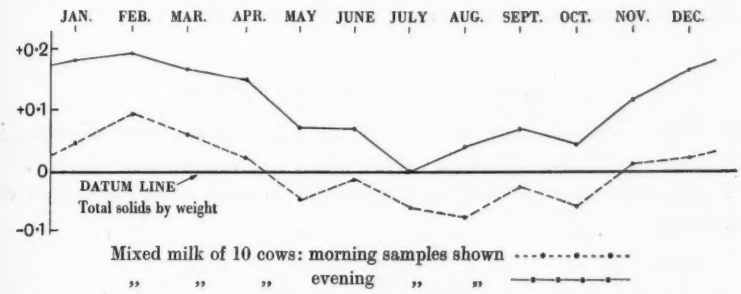


Fig. 1. Showing the errors of solids-not-fat percentages as calculated by Richmond's formula. Mean monthly errors for the period of 3 years.

Nitrogenous constituents.

The nitrogenous constituents are being further studied (9).

Butterfat.

The detailed examination and analysis of butterfat from the milk of this herd of cows was carried out by T. P. Hilditch and J. J. Sleightholme (10, 11).

The fat-soluble vitamins in butter from this herd (May 13, 1929), were investigated by Crawford, Golding, Perry and Zilva (12), and their variations as affected by season and nutrition are being studied at the Institute.

SUMMARY.

An investigation of the milk of a typical herd of Shorthorn cows kept under south of England conditions has been carried out.

The results of the first three years of the investigation are now recorded. The plan of the experiment, the history of the cows during the period, the management, feeding, milking, and milk yields are described and illustrated in Tables I and II.

Fat and solids-not-fat.

The daily fat tests and solids-not-fat estimations of the night's and morning's milk are set out in percentage distribution Tables III–VI. The differences between the fat in the night's and morning's milk, associated as it is with the 9- and 15-hour intervals between milking, is a notable feature in the composition of the milk.

The effect of season on these daily analyses is shown in the weekly average Tables VII–IX, which also record the weights of milk and the number of cows contributing.

Even in these weekly averages the percentage of fat in the morning's milk always falls below 3 per cent. in the spring.

This is in striking contrast with the solids-not-fat of the milk of these cows which only dropped below 8·5 per cent. under the exceptional conditions occasioned by an outbreak of husk in July-August, 1930.

Ash, calcium, phosphorus and nitrogen.

More detailed analyses for ash, calcium, phosphorus and nitrogen in samples of morning's milk taken once a week are given in Tables X–XV.

In these results uniformity of composition is more remarkable than the variations.

Rennet test.

Very definite alterations were, however, shown by the slowing down of the rennet test which occurs during the winter months when the time taken to coagulate the milk may be almost double the time taken in summer.

Relationship of estimated to calculated figures for solids-not-fat.

A comparison of the estimated non-fatty solids with the figures calculated from the fat and specific gravity indicated a seasonal relationship between the two sets of figures which is being further investigated.

This type milk has provided material for the still more detailed study of variations in the composition of the butter fat.

Our thanks are due to H. Hallett, A. Wagstaff and S. Watson who assisted in the analytical work and to B. Ridler for assistance in compiling the tables.

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(*MS. received for publication August* 29, 1932.)

637.12:576.851.5

49. THE INFLUENCE OF BACILLI OF THE COLON GROUP ON THE PRODUCTION OF ACID BY LACTIC STREPTOCOCCI IN MILK

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(With 4 Figures.)

In a previous paper(1), the effects of several species of organisms on the production of acid by lactic streptococci were described. It was shown that a mixture of lactic streptococci and colon bacilli growing in milk produced acid at a greater rate than the streptococci alone. In some experiments the quantity of acid produced as a result of the symbiosis of the two organisms was somewhat smaller than would be expected from a summation of their rates of acid production in milk when growing separately; in other experiments acid production was much enhanced as a result of symbiosis. It was suggested at the time that different results might be obtained if the colon bacilli were allowed to grow in the milk for some time before the addition of the streptococci. Such a procedure would more nearly approximate to the actual sequence of events in milk which is to be used for cheese manufacture. Colon bacilli are among the most common of the organisms which gain access to milk during its collection and handling, and they multiply in the evening milk for about 16 hours before the lactic streptococci are added in the form of starter at the beginning of the process of cheese manufacture (according to New Zealand practice). The present paper is an account of experiments designed to elucidate in greater detail the effect of colon bacilli on the production of acid in milk under conditions simulating those obtaining in the earlier stages of the process of cheese manufacture.

Three strains of colon bacilli were used: one of *B. lactis aerogenes* and two of *B. coli communis*. The culture of *Streptococcus lactis* was a single strain isolated from a "starter." The general procedure was similar to that described in the previous paper, *i.e.* sterile separated milk (200 c.c.) was incubated in flasks at 30° C., and all titrations were performed with $N/10$ NaOH on 10 g. of milk culture. The organisms were grown on agar plates from which the growth was washed with sterile water. In this way, even suspensions were obtained, suitable for distribution in the milk flasks in equal quantities. The experimental scheme was as follows:

In two flasks of milk the colon bacilli were sown in equal amounts from a well-shaken watery suspension, and allowed to grow at 30° C. for about

16 hours (usually overnight). The next morning the milk in two more flasks was inoculated in the same way from the same suspension of bacilli which had been standing at about 4° C. overnight. (Winslow and Falk (2) have shown that the number of colon bacilli in watery suspensions do not change appreciably under these conditions.) These two flasks were placed in the incubator at 30° C. for 3 hours. All the flasks were then transferred to the refrigerator at 4° C. until the main experiment could be commenced. The numbers of colon bacilli present in the milk samples at various stages were determined by dilution and growth in McConkey's broth. For several experiments the numbers ranges as follows:

Immediately after inoculation ...	100 millions to 100,000 millions per c.c.
After 3 hours at 30° C.	100 millions to 100,000 millions per c.c.
After 16 hours at 30° C. ...	100,000 millions to 100,000,000 millions per c.c.

When it was convenient to start the main experiment, the titratable acidity and the *p*H of the milks in the inoculated flasks was determined, and control flasks of the same separated milk were adjusted roughly to the same titratable acidity by addition of sterile lactic and sulphuric acids. The *p*H of the milk in these flasks was determined accurately. All the milk samples (plus two control samples of untreated sterile separated milk) were then inoculated with equal amounts of a suspension of lactic streptococci and transferred to the 30° C. incubator. Approximately 7 hours later the acidity of the milk in each flask was determined by titration. The results are best presented in the form of a graph, the increases in acidity being plotted against the *p*H values of the various milk samples at the time of inoculation of the streptococci. Each point is the average value from two similar flasks. The results obtained were remarkably constant in type throughout a large number of experiments, although, of course, the absolute values varied from experiment to experiment owing to differences in the number of lactic streptococci added and to variation in the times of incubation. Furthermore, since all three strains of colon bacilli gave similar results they can be dealt with under one head. Fig. 1 is a typical graph drawn from the results of one of the experiments. In the graph, the two points obtained with each method of treatment of the milk are joined by a straight line under the assumption that intermediate points would fall on the line. That this assumption is sufficiently near the truth for the present purpose was demonstrated by an experiment the results of which are graphed in Fig. 2. Samples of sterile separated milk were adjusted to various *p*H values by addition of lactic acid. Streptococci were added to the milks, as in the previous experiment, and the acidity increases were determined after incubation at 30° C. for 7 hours. In the graphs drawn from the results of the majority of the experiments the three straight lines corresponding to the three methods of treatment occupied the same relative positions. It is now possible to assess from a graph such as that given in Fig. 1 the effect of the previous growth of colon bacilli on the pro-

duction of acid in the milk by the lactic streptococci. The results given in milk samples to which lactic and sulphuric acids were added serve as a control on the effect of *p*H and of lactic acid and lactate ions on the production of acid by the streptococci.

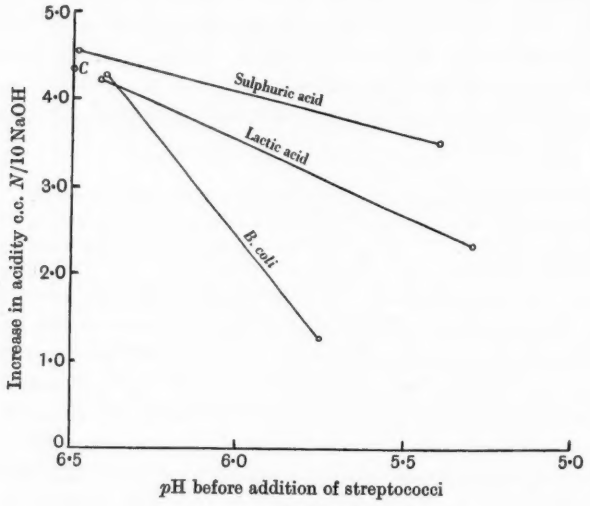

Fig. 1.

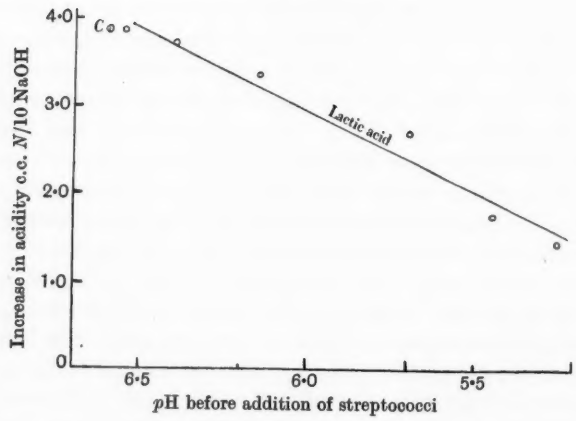

Fig. 2.

In the milk samples in which colon bacilli had been grown for 3 hours and the respective controls acidified with lactic and sulphuric acids, the acid production was not significantly different from that in the untreated controls. Sometimes there was a slight enhancement of acidity in the milk containing colon bacilli, but not in the majority of cases.

In the milk rendered more acid by the addition of sulphuric acid the amount of acid produced by the subsequent growth of streptococci was smaller than in the untreated milk. This is in accord with expectation, for the higher the acidity of milk at the time of inoculation, the less acid the streptococci will be able to form before their limiting *p*H is reached. It is also to be expected that lactic acid will have a more powerful inhibitory effect at a given *p*H, for the lactate ions and undissociated lactic acid molecules will militate against the formation of lactic acid in accordance with the law of mass action. Van Dam (3) favours the theory that undissociated lactic acid is a factor in the progressive decrease in rate of acid production in cultures of lactic streptococci.

The graph shows, however, that the previous growth of colon bacilli has a depressing effect greater than can be accounted for by either or both of the above factors. It is evident, therefore, that either the living colon bacilli react adversely on the streptococci while growing with them in the milk, or that during their previous growth the bacilli have formed some product other than hydrogen ions and lactic acid which has an additional inhibitory effect on the streptococci. It should be mentioned that in contradistinction to the results of the experiments described in the previous paper, the total amount of acid formed (by both colon bacilli and streptococci) in milks in which the colon bacilli have grown for 16 hours was considerably smaller than the amount formed by the streptococci in the control milks. In several of the present experiments extra flasks of milk were included in which the streptococci and colon bacilli were sown at the same time. In accordance with the findings previously reported (1), the milk in these flasks showed a greater increase in acidity than the control milks.

Further experiments were carried out in order to determine whether the specific inhibition caused by previous growth of colon bacilli was due to a chemical substance in the milk cultures or was a property of the living bacilli. The experiments already described were repeated with the sole modification that all the milk samples were pasteurised for 1 hour at 75° C. after the growth of colon bacilli and before the addition of the streptococcal suspension. Cultural tests showed that in most experiments all the bacilli had been killed by the pasteurisation. In a long series of such experiments it was found that the results were identical in type with those obtained in the first series where no pasteurisation was performed. Fig. 3 shows a typical graph drawn from the results of one of the experiments. A consideration of the graph leads to the conclusion that the inhibitory factor specific to the colon bacilli is some chemical substance produced by the organisms during their initial growth period, and stable to a moderate degree of heat.

A final series of experiments was performed in an attempt to provide a fuller picture of the variations in acid production from time to time in milk cultures of colon bacilli and streptococci. In these experiments the flasks of milk were prepared as before, but the acidity titrations were performed at

various intervals after the addition of the streptococci instead of at one arbitrarily chosen time. The flasks of milk were prepared exactly as in the previous experiments, and on these occasions the milk was not pasteurised after growth of the colon bacilli. Fig. 4 shows a graph plotted from a typical set of results,

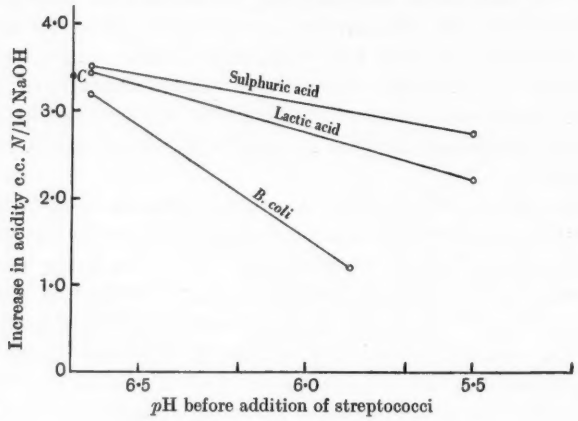

Fig. 3.

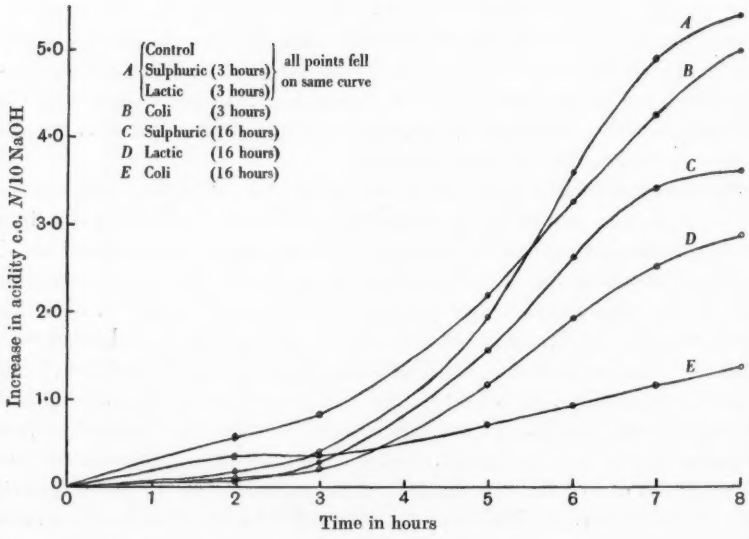

Fig. 4.

with increases in acidity as ordinates and intervals of times as abscissae. The fact that the *p*H values of the different milk samples varied at the time of inoculation of the streptococci cannot of course be indicated in the graph; but it should be remembered that all the curves marked 16 hours are plotted

from results of milk samples which had approximately the same *p*H when the streptococci were added. The same applies to the curves marked 3 hours. It is evident from the curves that all the 16-hour milk samples show smaller increases than the control throughout the period of observation, except in the case of the milk containing colon bacilli in which there is a slight enhancement of acidity during the first 3 hours. The milks in which colon bacilli had grown for only 3 hours show slightly greater acidity increases than the control during the earlier stages of growth of the streptococci. Thereafter, the inhibitory factors come into action and a diminished rate of acid production ensues. The results from milks adjusted with lactic and sulphuric acids to the same *p*H as the 3-hour coli flasks show no significant deviation from the control, and are represented as identical with the results from the control for the sake of simplicity in the graph. In all other respects the graph bears out the results of the previous experiments.

DISCUSSION.

The experiments described above prove conclusively that colon bacilli, when present in milk in numbers of the order of hundreds of millions per cubic centimetre, produce substances which inhibit the subsequent production of acid by lactic streptococci. Part of the inhibitory action is due to the lactic and other acids formed by the bacilli; but it is evident that other substances, at present undefined, play a part. The results of the experiments prove that part of the inhibition specific to the colon bacilli is due to definite chemical entities, not destroyed by heating at 75° C. There is no evidence as to whether they act by inhibition of the growth of the streptococci or by interference with metabolic activity.

In a consideration of the possibility of the phenomenon being encountered during the manufacture of cheese, the numbers of colon bacilli present in the milk used in the experiments must be considered. Even the numbers present in the milk incubated for 3 hours are much larger than those ordinarily encountered in milk supplies, and, in general, the experiments show that the inhibition of acid production does not assume marked proportions until the initial *p*H of the colon-infected milk is so low that the milk would immediately be recognised as abnormal in a cheese factory. It is unlikely, therefore, that the phenomenon would be encountered in a measure large enough to exert a significant influence on the progress of events in the cheese vat. The results of one or two experiments in which milk was infected with colon bacilli in numbers comparable with those encountered in practice confirmed this view, and, therefore, it cannot be claimed that any explanation is to be found here of the slowness of development of acidity which is sometimes experienced in the cheese vat.

It is possible, however, that the phenomenon has a practical significance in the maintenance of "starter" cultures. The milk in which "starters" are

sub-cultured from day to day is often infected with colon bacilli in numbers ranging up to one million per cubic centimetre. Although the bacilli are killed by pasteurisation before the inoculation of the milk with the starter, the inhibitory influence of their products must manifest itself in however small a degree. It is conceivable, therefore, that this adverse influence maintained from day to day may be one of the causes of the ultimate loss in vitality which all "starter" cultures suffer. This suggestion can be evaluated only in the light of further experiments.

SUMMARY.

1. Colon bacilli grown in milk for from 3 to 16 hours partially inhibit the subsequent formation of acid by lactic streptococci.

2. The inhibition is not entirely accounted for by the increased hydrogen-ion concentration nor by the lactic acid produced by the colon bacilli. Other substances which have a retarding influence on the streptococci are evidently formed in milk cultures of colon bacilli. The action of these substances is not significantly modified by heating at 75° C. for one hour.

3. The possible practical significance of the phenomenon is discussed.

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(*MS. received for publication May* 20, 1932.)

576.809.531

50. STUDIES IN THE METABOLISM OF THE LACTIC ACID BACTERIA

I. NITROGEN METABOLISM

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(With 6 Figures.)

Historical review.

In spite of apparently contradictory reports (1, 2) concerning the ability of the true lactic acid bacteria to utilise inorganic nitrogen, it is generally conceded that they are unable to grow without protein degradation products as a source of nitrogen. No bacteria apparently can attack pure native proteins (3), and of the mixed products of protein digests the micrococci, for example, prefer the amino acids and simpler peptides, only growing with proteose as a source of nitrogen in the presence of glucose, *i.e.* they are unable to use proteose as a source both of carbon and of nitrogen (4). Non-proteolytic bacteria do not break down albumoses and peptones (5), and the true lactic acid bacteria do not thrive with pure casein as a source of nitrogen, better growth being obtained when the casein is first split by pepsin, rennin, heat, or by other bacteria. The whole question of nitrogen availability must now be considered in relation to the recent developments concerning bacterial growth factors (6), and oxidation-reduction potential (7). It is impossible with the knowledge at present available to distinguish between a growth factor effect and an "available nitrogen" effect in a protein digest. There is evidence, however, that digests of pure proteins, such as casein, are greatly inferior to the crude digest present in the usual media, *e.g.* peptonised milk (8, 9). The known bacteriostatic effect of heated broths on delicate organisms such as some streptococci, has been shown to be due to the oxidation of thio-compounds, thus increasing the rH of the medium (10). If thio-compounds or other reducing agents be added after sterilisation, growth readily takes place. It is possible, therefore, that the presence of free-SH compounds in certain peptide fractions may control bacterial growth and that the availability of the nitrogen as such is not the determining factor.

Subject of investigation.

Experiments have been made to determine the availability of various amino acids and protein fractions for the true lactic acid bacteria. Strains of *S. lactis*, *L. plantarum* and *L. casei* isolated from Cheddar cheese, of *S. lactis*

and *S. cremoris* from Orla-Jensen's laboratory, and type cultures from the Lister Institute, London, have been used in this investigation.

Experimental methods.

The strains of bacteria used for this work were grown in 2 per cent. dextrose, 1 per cent. Hopkin and Williams peptone-yeast-extract-broth at 28° C., so that the cell suspension used would be in as vigorous a condition as could be uniformly obtained. Dilutions from 24-hour cultures were made in sterile saline as required. Aliquot portions were then pipetted into flasks of the experimental media adjusted to *p*H 6·6 ± 0·2. Cultures were incubated at 28° C. and portions withdrawn aseptically at intervals and titrated to phenolphthalein with *N*/9 caustic soda. These measurements are, strictly speaking, those of a metabolic change and not of growth. It was found, however, that growth as measured by turbidity, and fermentation as measured by acid production, always ran parallel.

Purity of cultures.

At the completion of each experiment slides were made and examined for possible contamination. In practice it was found that the acid curve and formol titration curve of pure cultures were so characteristic for any one species that contamination could always be detected by a sudden break in the curves, especially in the formol titration curve.

I. Experiments on the availability of simple nitrogenous compounds.

Attempts were first made to grow these bacteria in a medium containing only sugar, salts and amino acids. The amino acids were added roughly in the proportion in which they occur in casein, the percentage constitution of the medium being, dextrose 2, salt mixture 0·5, asparagine 0·2, alanine 0·2, tyrosine 0·1, glutamic acid 0·1, glycine 0·1, tryptophane 0·03, histidine 0·03, cystine 0·03 and valine 0·03. The amino acids were the purest obtainable commercially, and the percentage composition of the salt mixture was NaCl 36, Na_2HPO_4 36, KH_2PO_4 18, $MgSO_4$ 4·5, $CaCl_2$ 4·5 and $Fe_2(SO_4)_3$ 1.

No growth was obtained upon inoculating tubes of this medium with one loopful of a broth culture or 1 c.c. of a one in ten dilution in saline. Addition of ammonium citrate and of allantoin (providing ammonia and a purine base as sources of nitrogen and citrate as a source of carbon) also failed to induce growth. The cultures were examined for growth over a period of 6 months.

It has been shown, however, that some amino acids, particularly tryptophane, glycine and phenylalanine, inhibit growth, and that others favour growth, *e.g.* taurine, aspartic acid and alanine in the case of *Gonococcus* and similar delicate organisms (11). The medium as described above was therefore diluted to five times its volume in order to reduce the concentration of these

inhibitory amino acids and 0·1 per cent. taurine (prepared from taurocholic acid (12)) and 0·2 per cent. alanine added. This modified medium did not permit growth. In order to ascertain whether the absence of growth was due to the non-availability of the sources of nitrogen present or to toxic factors, peptone (Hopkin and Williams) was added to the synthetic medium. Growth readily took place in this combined medium.

It appears, therefore, that the true lactic acid bacteria are unable to assimilate pure amino acids alone, and that their growth on casein digests is due to some or all of the following factors: (i) the peptone fractions, (ii) unknown amino acids, (iii) substances of the nature of "growth factors," and (iv) substances capable of poising the oxidation-reduction potential.

The work of Rose (13) indicates that unrecognised amino acids exist which are essential for animals, since rats did not grow on a complete mixture of known amino acids. The lactic acid bacteria may thus resemble the higher animals in this respect.

II. The effect of yeast extract and marmite as typical sources of growth factors on the assimilation of simple nitrogen compounds.

Media prepared for these experiments contained 2 per cent. dextrose, 0·2 per cent. marmite (or 0·1 per cent. dried brewers' yeast) and ammonium salts or amino acids to make a nitrogen concentration of 0·14 per cent.

The effect of adding various simple nitrogen compounds is shown in Fig. 1. The chief interest of these results lies in the great stimulation of growth ob-

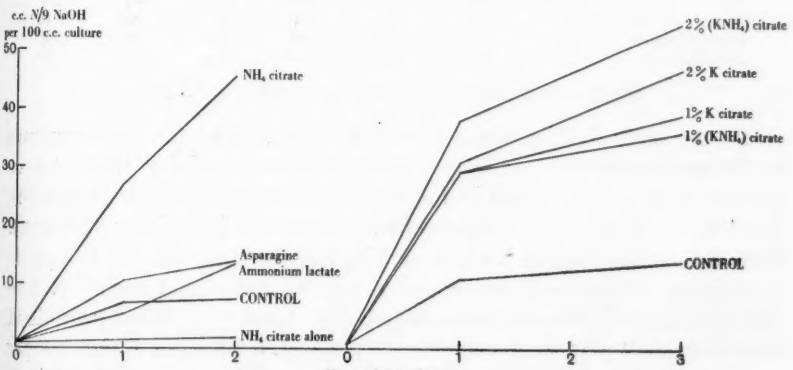

Fig. 1. Showing the effect of adding various sources of nitrogen to the basal medium 2% dextrose and 0·2% marmite (as a source of "growth factors").

served when ammonium citrate was added. This was found to be solely due to the buffering effect of the salt, as further experiments in which ammonium and potassium citrate were added to duplicate cultures showed that the anion

was responsible for the stimulation of growth. No evidence could thus be obtained that these bacteria could utilise inorganic nitrogen in the presence of yeast extract, even when a large inoculum containing small amounts of peptone was added. Experiments were then made to investigate the nature of the nitrogen sources essential for the growth of the lactic acid bacteria.

III. The relation between the rates of fermentation and the nitrogen distribution in different peptone media.

It has been shown that the different commercial peptones vary enormously in their nitrogen distribution (14), some consisting largely of "protein" and others mainly of the lower peptides and amino acids. If the bacteria under investigation utilise preferentially any of the arbitrarily defined groups (proteose, peptone, polypeptide and amino acids) then, other conditions (such as the presence of possible growth factors) being equal, the fastest growth should be obtained with the peptone containing the largest amount of that fraction. For the purpose of investigating this possibility, media were made as follows: dextrose 2 per cent., peptone 1 per cent., salt mixture (as before) 0·5 per cent.

Four peptones, Witte's, Bactoproteose, Bactopeptone and Fairchild's were used; their chemical analyses are given in Table I. Typical experimental

Table I. *Analytical data of commercial peptones.*

		Nitrogen distribution					Formol titration per 10 c.c. medium
Peptone	N %	Protein N	Non-protein N	Poly-peptides	Van Slyke	Sørensen	
Witte's	14·8	9	5·6	2·6	2·7	0·7	1·4
Bactoproteose	12·0	5	6·7	2·6	4·4	1·3	2·0
Bactopeptone	14·0	0·6	12·8	8·0	4·8	2·1	2·2
Fairchild's	12·6	2·8	10·0	5·0	4·8	2·9	5·0

These figures (except the last column) are taken from ref. (13).

results are shown in Figs. 2 and 3. It will be seen from these measurements of the comparative rates of fermentation in media containing different proportions of protein, etc., that all the streptococci behave similarly in that the greatest rate of growth is obtained with Bactoproteose (containing 5 per cent. protein N and 1·3 per cent. amino acid N) and the least with Bactopeptone (containing 2·1 per cent. amino acid N and 0·6 per cent. protein N). The lactobacilli (Fig. 3) behave quite differently, the fastest growth being obtained with Fairchild's peptone (containing 2·9 per cent. amino acid N and 2·8 per cent. protein N) and the slowest with Witte's (containing 9 per cent. protein N and 0·7 per cent. amino acid N). The superiority of Fairchild's peptone in the latter case is very marked. (Some of these experiments were made in media in which the peptone concentration was such that all media possessed the same nitrogen content, but without any effect on the results already stated.)

This difference in behaviour of the streptococci and lactobacilli may be explained by the hypothesis that the former prefer the higher and the latter

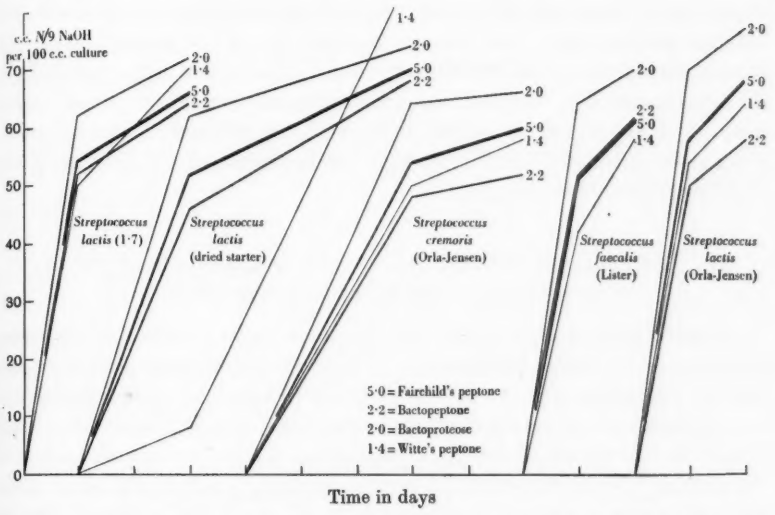

Fig. 2. Showing the effect of amino *N* content of various peptones on the rate of growth of streptococci. The figures give the formol titration (c.c. *N*/9 NaOH per 10 c.c.) of medium.

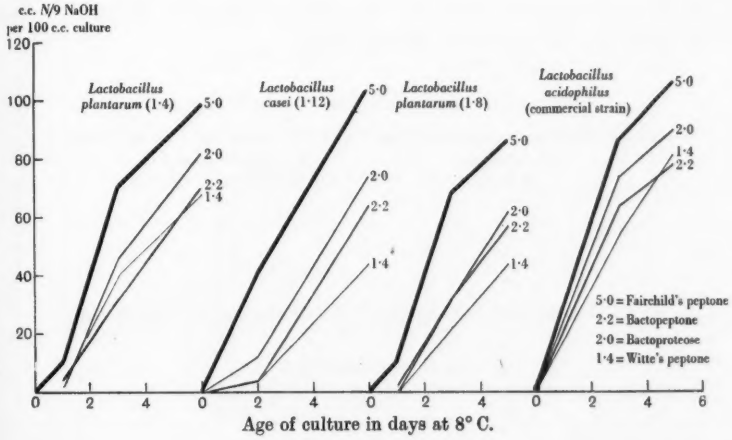

Fig. 3. Showing the effect of amino *N* content of peptones on the rate of growth of lactobacilli.

the lower fractions of the digest. It is obvious, however, that since the sources of the digests were not controlled, no conclusions can be drawn from these experiments. Thus the differences observed may be due to the fact that different proteins were used for the manufacture of the peptone, with the consequent differences in growth factor and —SH content, etc. Accordingly the experiments were repeated, using casein digests from which portions were removed at different times corresponding with the different extents of breakdown of the commercial peptones. Such media eliminated the differences due to different protein sources, etc.

IV. The relation between the extent of enzymic degradation of casein and the rate of fermentation.

Peptic digests were prepared using various types of casein, Lait-Proto, Burgoyne and Burbidge's fat free, B.D.H. light white, and casein separated from milk by precipitation at the iso-electric point (hydrochloric acid method (15)), and also different specimens of pepsin. Since four types of casein were used it is evident that the results are not dependent on the method of preparation of the protein. The digests were prepared by adjusting a mixture of 50 g. casein and 5 g. pepsin in 1000 c.c. tap water to a *p*H of 1·6, using thymol blue as an indicator. These mixtures were incubated at 37° C., in some cases in the presence of chloroform. Portions of the digest were removed at various times and incorporated in the media to give a concentration of 1 per cent. original protein. The media were prepared, inoculated and tested as described earlier in the paper. The results obtained are shown in Figs. 4 and 5, and the analytical data of the media given in Table II. The ordinates show the acidity developed and the abscissae the age of the casein digest.

Table II. *Analytical data of casein digests.*

Age of digest in days	Formol titration c.c. *N*/9 NaOH per 10 c.c. digest
Exp. 1 (Fig. 4). Burgoyne and Burbidge's fat-free casein, B.D.H. pepsin.	
0	0·8
2	2·8
7	4·0
17	4·2
35	5·4
Exp. 2 (Fig. 5). Lait-Proto casein. B.D.H. pepsin (different specimen).	
0	3·8
7	7·3
18	8·0
30	11·0
60	15·0

It will be seen that the streptococci differ from the rod forms as in the previous experiments, the former growing most quickly in media containing casein digested for about 15 days and the latter in those media in which the casein has been digested for longer periods (up to 60 days).

These experiments eliminate as far as possible the possibility that the differences are due to different proteins, growth-factor content, etc., but there remains the possibility that the inhibition of the growth of the streptococci

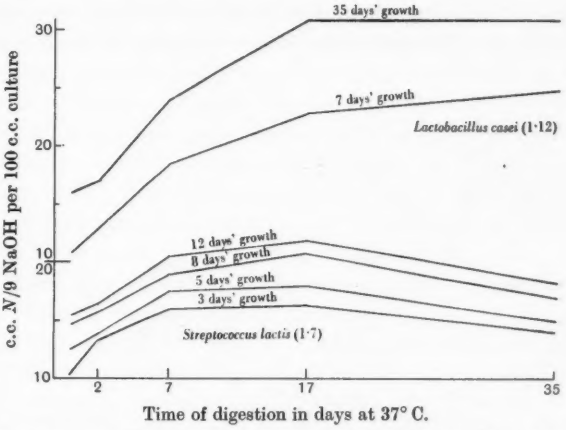

Fig. 4. Showing the relation between the age of peptic digestion of casein and the rate of fermentation.

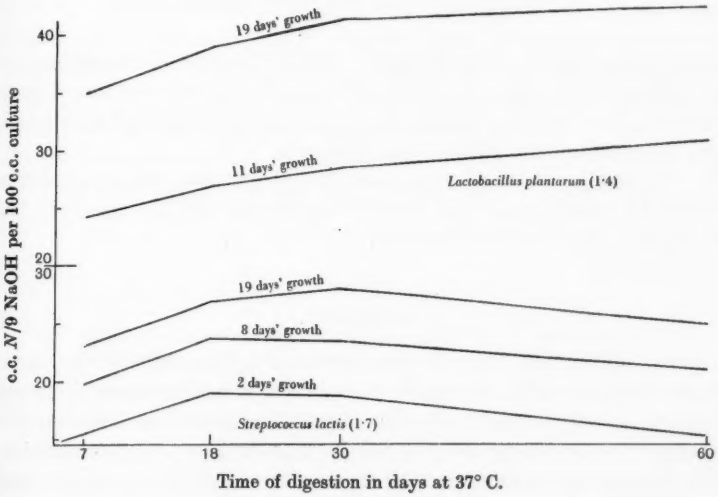

Fig. 5.

in the older digests is due to the increase in concentration of certain amino acids (11). Assuming that the liberation of the amino acids from casein is uniform with respect to the different amino acids, and that 25 per cent. of the protein is converted into amino acids (a figure considerably higher than

is realised in practice) it can be calculated that the "toxic" amino acids would be present in the final medium in the following concentrations: tryptophane 0·005 per cent., glycine nil, phenylalanine 0·008 per cent., cystine 0·00025 per cent. It is thus evident that the toxicity of these amino acids cannot explain the falling off in rate of growth of the streptococci in the older casein digests. The same phenomenon was observed in media prepared

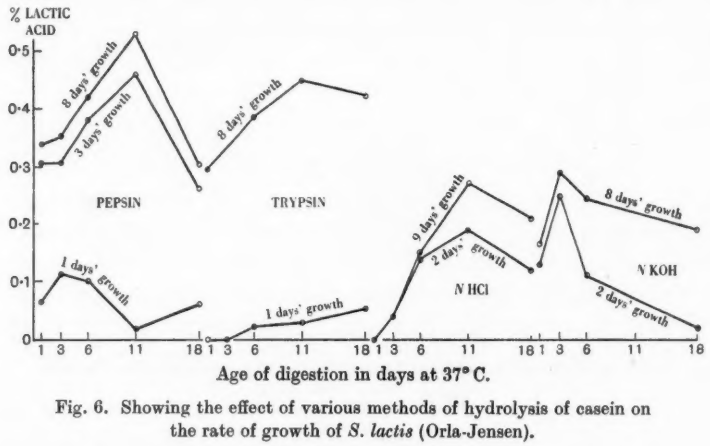

Fig. 6. Showing the effect of various methods of hydrolysis of casein on the rate of growth of *S. lactis* (Orla-Jensen).

from casein by digestion with pepsin, trypsin (at *p*H 8·5), *N* hydrochloric acid and *N* sodium hydroxide at 37° C. (Fig. 6). The small amount of growth obtained in the media containing casein hydrolysed with alkali may possibly be due to racemisation of the protein products.

It may also be of interest to record the observation that yeast extracts hydrolysed by alkali (*e.g.* at *p*H 9 for half an hour at 100° C.) have been found to be highly toxic to certain bacteria.

Discussion.

The possibility that the differences observed in the growth of the bacteria used are due to the differing distributions of the various non-protein nitrogen compounds is only one of many hypotheses. The known predilection of the rod forms for micro-aerophilic conditions suggests that the concentration of —SH groups may contribute to the superiority of Fairchild's peptone and of the most extensively peptonised casein media, although when the sulphur content of some of the peptones were determined by the perhydrol method (16), no correlation was found between the values for sulphur and the rate of growth of the lactobacilli.

It is important to note that in all cases the rates of growth were compared in the logarithmic phase, *i.e.* before inhibition by acidity had taken place.

That the buffer values of the peptones were not responsible for the differences observed, is clearly shown by the fact that the most extensively digested, and hence the most powerfully buffering peptones were not the best for the streptococci, which are much more acid sensitive than the lactobacilli.

The following tentative suggestions as to the reasons for the predominance of certain bacterial groups under working conditions may be made.

The condition and concentration of protein in foodstuffs is one of the most important factors controlling the flora. Thus protein matter (such as meat) liberating sulphydryl groupings on hydrolysis, readily gives an almost entirely putrefactive flora of anaerobes on standing, because this type of organism is best fitted to obtain energy under these conditions. Similarly, the lactic acid flora predominate in hard cheese on account of the fermentable sugar. These bacteria are, however, very sensitive to the sources of nitrogen in a medium, since, as shown in this paper, they are entirely dependent on intermediate fractions or unknown amino acids.

The fact that digestion of protein beyond a certain point results in an inhibition of streptococcal growth suggests that the degradation of casein in the ripening of the cheese may well be a factor in the control of the flora, especially as the rod forms grow best when the amino nitrogen concentration of the medium is high. Many methods of preparing these media have been described from time to time, but all bacteriologists appear to experience trouble on occasion, some batches of digest failing completely to support growth. From the evidence presented in this paper it appears to be advisable to adjust the time of digestion according to the type of organism under culture. It has recently been shown (17) that some proteins occurring in milk, etc. (*e.g.* lactalbumin) exist as precursors, of low molecular weight, of the substances precipitated in the "purification" process. This may be one reason why digests of purified casein are poor in comparison with milk itself.

Summary.

1. The true lactic acid bacteria cannot grow in a medium in which the sole source of nitrogen is an amino acid mixture or an ammonium salt.

2. The presence of growth factors (yeast extract, marmite and peptones) does not permit the utilisation of this "simple" nitrogen.

3. The streptococci grow fastest with those peptones containing the greatest amount of the higher fractions, Bactoproteose being superior to the other peptones tested; the lactobacilli, however, show a considerably greater fermentation rate with those peptones containing most amino nitrogen, Fairchild's being the best. Similarly, the streptococci thrive best upon a peptic casein digest which is about 15 days old, further digestion impoverishing the growth; the lactobacilli, however, grow better the longer the digestion. The significance of these observations is discussed from the aspects of bacterial

metabolism, the sequence of flora in ripening cheese, and the preparation of casein digest media.

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(*MS. received for publication August* 29, 1932.)

576.809.33:637.12

51. THE PLATE METHOD OF ESTIMATING THE BACTERIAL CONTENT OF MILK—THE LIMITATIONS OF PROCEDURE IN COMMON USE, WITH SUGGESTED IMPROVEMENTS

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(With 3 Figures.)

The plating method of estimating the bacterial content of milk is the most important means of determining the number of living organisms present, and is the one most frequently employed. It is well recognised, however, that accurate counts cannot be obtained by this method, since considerable errors may be involved through the difficulty of ensuring the separation of chains and clusters of organisms so that each organism is represented by a separate colony, through the inability of some classes of bacteria to grow on the medium employed, and through the fact that the presence of one group of organisms may inhibit the growth of others—particularly on crowded plates. It appears that the difficulties in obtaining uniformity of counts for duplicate tests may be increased in the case of milk because this fluid is heterogeneous in nature, and the bacterial contaminants are liable to occur not only as groups or individuals freely dispersed in the liquid but also in association with matter in suspension, especially the clusters of fat globules. Consequently the bacterial counts are likely to vary with the extent to which the clusters of fat globules are broken up or aggregated by the manipulations.

It has, in fact, been found that in many instances there is a great variation in the results obtained from duplicate examinations of the same sample of milk, especially where the tests are performed by different workers and in different laboratories. This is undoubtedly due in part to differences in the technique and the medium employed, and also to differences in manipulative skill. On the other hand it appeared probable that considerable errors might also be due to the limited number of plates examined. The object of the present work, therefore, was to examine the general problem of the reliability of a limited number of parallel plate counts as an index of the viable bacterial content of samples of milk. This involves the question of the extent of variation between individual counts when large numbers of plates are prepared from the same sample under similar conditions.

Throughout the work, except where otherwise stated, the pipettes used were calibrated to *deliver* 1 c.c., and plates of standard size (10 cm. diameter) were employed. The counts were made, after incubation at 37° C. for 48 hours,

by naked eye examination in a good window light. Details of the methods of making dilutions are dealt with separately in the following section.

A SIMPLIFICATION OF THE OFFICIAL METHOD OF PLATING.

The procedure prescribed by the Ministry of Health (1) for the estimation of the bacterial content of milk by the plating method is to make dilutions of (*a*) 1/10, (*b*) 1/100 and (*c*) 1/1000. Dilution (*a*) is prepared by adding 10 c.c. of milk to 90 c.c. of sterile water; dilution (*b*), by adding 10 c.c. of dilution (*a*) to 90 c.c. of sterile water; and dilution (*c*), by adding 10 c.c. of dilution (*b*) to 90 c.c. of sterile water. Agar plates are then poured from the respective dilutions, 1 c.c. of inoculum being used for each plate. Where a large number of samples are tested, this method entails the use of large quantities of media and much apparatus. The question therefore arose as to whether equally satisfactory results could be obtained using a simpler and less expensive method, and the following considerations regarding the dilutions presented themselves.

In making the plate or colony counts, plates prepared from dilution 1/10 are only employed in the case of samples containing less than 3000 bacteria per c.c. But these samples are of such a high standard of purity that it is questionable whether, in the routine examination of milk, there is any practical advantage to be gained by a more accurate count than that afforded by the 1/100 dilution plates. Consequently the 1/10 dilution plates are as a rule unnecessary.

Most of the milk samples received in the writer's laboratory are less than 24 hours old when tested, the age of the evening milk samples being 20–22 hours and that of the morning milk samples 8–10 hours. In many cases they come from farms where great care is taken in the production and treatment of milk. The majority of the samples therefore contain less than 30,000 bacteria per c.c., and so plates prepared from the 1/100 dilution are generally employed in making the counts. In samples containing a greater number of bacteria, even although there are so many colonies on these plates as to make only an approximate estimate possible, the plate counts in conjunction with the coliform test give a very good indication of the degree to which milk of this age has been contaminated, and the temperature at which it has been kept.

It was evident, then, that the existing method would be simplified and the amount of work reduced if the 1/10 dilution were omitted and the 1/100 dilution made by adding 1 c.c. of the sample of milk to 99 c.c. of sterile water. A number of samples were therefore examined by both methods, five, and in a few samples ten, parallel plates being employed. The milk and the 1/100 dilution bottle were subjected to the same amount of agitation as in the official method, *i.e.* they were shaken 25 times, but with a number of the later samples the 1/100 dilution bottle was shaken 40, and in a few cases 100 times. The object of this additional shaking was to ensure thorough mixing

of the inoculum with the sterile water. A later experiment, however, proved that this was unnecessary.

The results obtained by the two methods correspond very closely (see Table I). The differences between the mean plate counts are slight and in most cases less than the standard deviations. They are therefore of no significance.

The proposed method has the advantage of being not only simpler, but it requires less apparatus (fewer dilution bottles and pipettes), and results in a saving of time and labour. Less time is spent in performing the necessary

Table I. *Comparison of counts obtained by official method with those obtained by author's method.*

	Official method			Proposed method		
Sample No.	Mean plate or colony count	S.D.	Coefficient of variation %	Mean plate or colony count	S.D.	Coefficient of variation %
83	42	1·5	3·6	42	1·4	3·4
84	570	47·4	8·3	595	35·1	5·9
85	144	2·4	1·7	117	5·3	4·6
86	86	1·8	2·1	85	2·8	3·3
87	189	23·7	12·5	156	22·9	14·7
88	1068	69·1	6·5	1015	85·0	8·4
89	67	2·0	2·9	88	4·3	4·9
90	106	3·0	2·9	112	9·0	8·0
91	55	11·0	20·1	54	7·5	13·8
93	10	1·8	18·3	9	5·5	60·8
94	592	34·5	5·9	560	47·8	8·5
95	58	4·8	8·2	59	7·0	11·8
96	17	3·6	20·9	15	3·1	20·8
97	56	6·2	11·2	55	5·4	9·9
98	123	3·1	2·5	124	19·8	16·0
99	170	2·9	1·7	111	7·0	6·3
100	19	2·1	11·2	25	30·0	120·0
101	8	2·0	23·8	5	2·3	45·8
102	132	6·8	5·1	115	16·9	14·7
103	69	6·5	9·3	75	24·6	32·8
104	23	2·3	9·9	23	6·3	27·2
105	213	16·9	7·9	191	6·5	3·4
106	155	16·2	10·4	159	16·2	10·2
108	151	22·8	15·1	139	16·0	11·5
109	23	6·2	27·0	24	4·8	20·0
110	13	4·8	37·0	23	4·6	20·0
111	22	7·8	35·2	20	3·0	15·2
112	35	3·4	9·5	41	5·9	15·5
113	22	5·5	25·1	13	2·4	17·5
249	37	3·5	9·4	35	4·2	11·9

manipulations, and in cleaning, preparing, and sterilising apparatus, of which much less is required. The simplicity of the method renders it less liable to error. Finally, a considerable reduction in the cost is effected.

As regards the higher dilutions, in the official method for the estimation of the bacterial content of milk, the plates for the 1/1000 dilution are prepared by taking as the inoculum for each plate 1 c.c. of dilution (*c*), 1/1000. It would considerably simplify the method and reduce the work and the amount of apparatus required, if the preparation of dilution (*c*), 1/1000, were omitted and the 1/1000 dilution plates poured by using as the inoculum 0·1 c.c. of dilution (*b*), 1/100. Preliminary experiment showed that this could not be

effected with sufficient accuracy by measuring 0·1 c.c. with the 1 c.c. graduated pipette. Accordingly the experiment was repeated, using a pipette which is designed to deliver 0·1 c.c., the last drop being expelled. Sets of five parallel plates were poured as before. The results are given in Table II. It will be seen that in all samples there is very close agreement between the corresponding means and standard deviations of the 1/1000 dilution plate counts.

The method proposed above is simpler than the official one and requires fewer dilution bottles and pipettes. At the same time the results show a close correspondence with those given by the official method. When estimating the

Table II. *Five plate counts.*

	Dilution 1/100			Dilution 1/1000					
				Official method			Proposed method		
Sample No.	Mean	S.D.	Coefficient of variation %	Mean	S.D.	Coefficient of variation %	Mean	S.D.	Coefficient of variation %
225	10	0·8	7·8	1	0·6	64·5	1	0·8	77·5
233	20	1·6	7·8	2	3·1	154·9	2	1·9	97·0
226	37	2·6	7·0	3	2·5	81·7	3	2·1	68·8
236	54	2·5	4·6	7	1·2	16·6	7	0·8	11·1
238	52	3·0	5·7	6	0·8	12·9	7	1·2	16·6
240	91	2·7	3·0	9	1·3	14·3	8	1·2	14·5
239	83	1·7	2·0	8	1·2	14·5	8	1·7	21·0
224	91	3·4	3·7	10	0·8	7·8	10	0·8	7·7
241	125	4·8	3·8	11	0·6	5·9	11	0·8	7·0
230	132	5·9	4·5	12	0·8	6·5	12	0·8	6·5
246	110	5·0	4·6	14	1·5	11·1	13	2·3	17·8
223	68	12·1	17·9	14	2·2	15·7	14	2·6	18·4
244	118	4·4	3·7	14	1·8	12·9	15	1·0	7·0
245	146	4·5	3·1	15	2·6	17·2	15	1·5	10·3
231	227	8·1	3·6	14	0·8	5·5	16	1·2	7·3
242	266	13·6	5·1	23	1·0	4·5	22	1·0	4·7
248	262	12·2	4·6	27	1·8	6·8	26	2·5	9·7
229	338	17·8	5·3	32	1·2	3·6	31	0·6	2·1
237	360	7·1	2·0	38	1·7	4·4	38	1·5	4·1
243	354	13·2	3·7	51	3·4	6·6	48	1·7	3·5
227	338	17·8	5·3	51	1·2	2·3	50	2·2	4·4
234	442	8·5	1·9	71	1·0	1·5	71	1·2	1·6
228	813	39·0	4·8	92	2·3	2·5	93	2·5	2·6
232	1495	70·6	4·7	118	3·1	2·6	121	1·7	1·4
247	1024	8·0	0·78	121	3·4	2·8	124	4·3	3·5
235	4497	100·4	2·2	718	39·0	5·4	733	44·2	6·0

bacterial content of milk, it is therefore possible to effect a great saving in time, labour and cost, by omitting the 1/10 dilution bottles, and preparing by the proposed methods the 1/100 and 1/1000 dilution plates.

The reliability of the plating method.

In estimating the bacterial content of milk by the plating method, if the organisms are unevenly distributed in the milk and in the dilution water, there will be a variation in the number present in each cubic centimetre of inoculum used for the plates and in the number of colonies formed on the plates. An error will, therefore, occur which will vary according to the degree

of uniformity in which the organisms are distributed. The error will be less if a number of parallel plates are made, so that the final estimate of the bacterial content is based, not on the results obtained from one plate, but from two or more.

Numerous experiments have been carried out to determine the extent of this error. In many cases parallel plates from the same sample were made, five, ten or twenty parallel plates being prepared. The plates were numbered in sequence as they were poured, so that a comparison could be made of the colony counts of the first two, the first five, and so on. In carrying out these experiments 1/100 dilution plates were examined, prepared at first by the official method, and latterly by the new method previously discussed. The inoculum was placed directly in the Petri plate, agar was added and the two then mixed. The distribution of the colonies on the plates showed that the agar and the inoculum had been satisfactorily mixed. Care was taken to maintain uniformity in the manipulations. The time that elapsed from start to finish did not exceed 15 min. A fresh pipette was taken for each dilution and also for each series of parallel plates prepared from any one dilution. It is noteworthy that the first plate count of any one series of parallel plates did not differ significantly from the successive plate counts, nor was there any correlation between the individual plate counts, and the order in which they were poured, as would have been found if the pipette effect, emphasised by Albus (2) in his criticism of Prescott and Parker (3), was of real importance.

The plates were examined after 48 hours incubation at 37° C. Where less than 500 colonies were found on a plate, all the colonies were counted; where more than 500 colonies were found, the plate was divided into segments, and the colonies on two or more of these counted. The size of the segments depended on the number of colonies on the plate, but generally the colonies on a half or a quarter of the plate were counted. A few plates on which a very large number of "pin point" colonies appeared were discarded.

In order to compare the variations in bacterial count in a number of parallel plates prepared from the same sample, use has been made of the arithmetic mean of the parallel plate counts and of the standard deviation (4) as calculated by means of the formula:

$$\text{S.D.} = \frac{\Sigma d^2}{n} \times f,$$

where d = the deviation from the arithmetic mean, n = the number of observations, and f = the correcting factor for low values of n (5). In order to compare the variation in series of plates, each series prepared from a different sample of milk, it is clear that absolute figures such as the mean and the standard deviation are useless because different samples may differ widely in their bacterial content. Accordingly for this purpose it is necessary to express the variation of each series as a function of the mean. Use was therefore made of a measure of relative dispersion, Pearson's Coefficient of Variation (6), which

is the ratio of the standard deviation to the arithmetic mean, expressed as a percentage, and calculated by means of the formula:

$$\text{coefficient of variation} = \frac{100 \times \text{standard deviation}}{\text{arithmetic mean}}.$$

The coefficient of variation obviously cannot be employed for the comparison of the variations in duplicate plate counts, as in such cases the standard deviation is indeterminate.

Samples with less than 50 colonies per plate may give very high coefficients of variation as a result of the low values of the means. For this reason, in preference to the coefficients of variation for these samples, the standard deviations have been used as a basis for comparison.

Table III is a frequency table of the standard deviations of the means of 20 parallel plate counts of samples giving less than 50 colonies per 1/100 dilution plate. It will be seen that the standard deviation in 51 per cent. of the samples was less than 5·0, in 98 per cent. it was less than 10·0, and in no instance did it exceed 15·0. The mean of the standard deviations was 4·8. These results show that in samples which give mean colony counts of less

Table III. *Frequency table of the standard deviations of samples giving colony counts of less than* 50 *on the* 1/100 *dilution plates* (20 *parallel plates*).

S.D.	Frequency	Frequency, expressed as a percentage of the number of samples
0 – 4·4	26	51
4·5– 9·4	24	47
9·5–14·4	1	2
Over 14·4	—	—

Total number of samples = 51; mean of standard deviations = 4·8.

than 50 on the 1/100 dilution plates, there is fairly close agreement between the parallel plate counts. The deviations found are not high from the biological standpoint. They represent variations in bacterial counts which are of no practical significance in milk analysis.

Table IV is a frequency table of the coefficients of variation of the counts of 20 parallel plates in the case of 69 samples giving 50 or more colonies per 1/100 dilution plate. It will be seen that 51 per cent. of the samples have coefficients of variation of over 24·4 per cent. The parallel plate counts of such samples vary considerably. This variation is so great, even in the case of a series of plate counts with a coefficient of variation of only 25 per cent., that it constitutes a serious source of error in the estimation of the bacterial content of milk by the official method, even where two plates are used for each dilution. For example, individual plates belonging to a parallel series with a mean count of 250 and a coefficient of variation of 25 per cent. will yield counts ranging from 188 to 312, so that if only two plates have been used and their colony counts are at the upper limit of the range the bacterial content may be estimated as 31,200 instead of 25,000. Similarly if the colony

counts are at the lower limit of the range the bacterial content may be estimated as 18,800. In the case of samples with higher coefficients of variation the range of variation of parallel plate counts is much greater.

The wide range of variation between parallel plate counts which may occur in the case of samples whose mean plate counts are 50 or over, can be clearly brought out as follows. The percentage difference between the mean of the two lowest plate counts and that of the two highest for each sample is calculated by means of the formula:

$$\text{percentage difference} = \frac{(X_1 - X_2) \times 100}{Y},$$

where X_1 = the mean of the two highest plate counts, X_2 = the mean of the two lowest plate counts, and Y = the mean of the 20 plate counts.

Table IV. *Frequency table of the coefficients of variation of the samples giving colony counts of* 50 *and over, on the* 1/100 *dilution plates* (20 *parallel plates*).

Coefficients of variation %	Frequency	Frequency, expressed as a percentage of the number of samples
0 – 4·4	—	—
4·5– 9·4	6	8·7
9·5–14·4	10	14·5
14·5–19·4	11	15·9
19·5–24·4	7	10·1
24·5–29·4	10	14·5
29·5–34·4	9	13·0
34·5–39·4	4	5·8
39·5–44·4	2	2·9
44·5–49·4	—	—
49·5–54·4	1	1·5
54·5–59·4	2	2·9
59·5–64·4	1	1·5
64·5–69·4	2	2·9
69·5–74·4	1	1·5
Over 74·4	3	4·3
	Total 69	

The results so obtained from samples whose mean plate counts are 50 or more are expressed in the form of a frequency table in Table V. In approximately 70 per cent. of these samples the percentage difference is 50 per cent. or more, in 26 per cent. of the samples it is 100 per cent. or more. The significance of such differences between plate counts in samples of which the average bacterial content is 32,000 or 100,000 is shown in Table VI. This table gives the number of bacteria estimated from plate counts which differ by 25, 50 and 100 per cent. It is evident from Tables V and VI that if only two plates are poured for each dilution and the average bacterial content of the milk is over 5000 as estimated on the 1/100 plates there is a possibility of a serious error arising when making bacterial estimations of 70 per cent. of the samples, due to the fact that both plate counts may happen to fall at the higher or the lower limit of the range of variation. The probability of this occurring is 1 in 95. A still greater error would be possible if bacterial counts were to be judged by single plates of each dilution.

From a consideration of the various points discussed above it therefore appears clear that in a very high proportion of samples, of which the average bacterial content is 5000 or more (*i.e.* the mean plate count is 50 or more), the plating method of estimating the bacterial content of milk may yield highly misleading results if only one, or even two, plates are poured for each dilution.

Table V. *Frequency table of the percentage difference between the means of the two lowest and the two highest counts. Samples with mean plate counts of 50 and over.*

Percentage difference between the mean of the two lowest plates and that of the two highest	Frequency	Frequency, expressed as a percentage of the number of samples
0 – 9·4	1	1·5
9·5– 19·4	2	2·9
19·5– 29·4	6	8·7
29·5– 39·4	3	4·3
39·5– 49·4	9	13·0
49·5– 59·4	7	10·1
59·5– 69·4	5	7·2
69·5– 79·4	3	4·3
79·5– 89·4	7	10·1
89·5– 99·4	8	11·6
99·5–109·4	3	4·3
109·5–119·4	1	1·5
119·5–129·4	2	2·9
129·5–139·4	1	1·5
139·5–149·4	1	1·5
149·5–159·4	1	1·5
159·5–169·4	3	4·3
169·5–179·4	—	—
179·5–189·4	—	—
189·5–199·4	3	4·3
Over 199·4	3	4·3
	Total 69	

Table VI.

Percentage difference between the mean of the two lowest plates and that of the two highest	Average bacterial content	Mean of two lowest counts	Mean of two highest counts
25	32,000	28,000	36,000
	100,000	87,500	112,500
50	32,000	24,000	40,000
	100,000	75,000	125,000
100	32,000	16,000	48,000
	100,000	50,000	150,000

The question arises as to whether more reliable results can be obtained by the use of more than two plates for each dilution. As it was the practice to number the plates in sequence as they were poured, it was possible to calculate the means of the first two, five and ten and the 20 parallel plate counts. A comparison of these means, obtained from 120 samples, is expressed graphically in Figs. 1, 2 and 3. A logarithmic scale has been employed so as to cover the range satisfactorily. If the mean count of 20 plates is taken as

the standard, it is evident that the results of five and ten plate counts are much more reliable than those of two.

The actual differences between the mean of the first two, five and ten plate counts respectively and that of the 20 plate counts were calculated, and also the percentage differences, the latter being determined from the formula:

$$\text{percentage difference} = \frac{(M_{20} - M_x) \times 100}{M_{20}}.$$

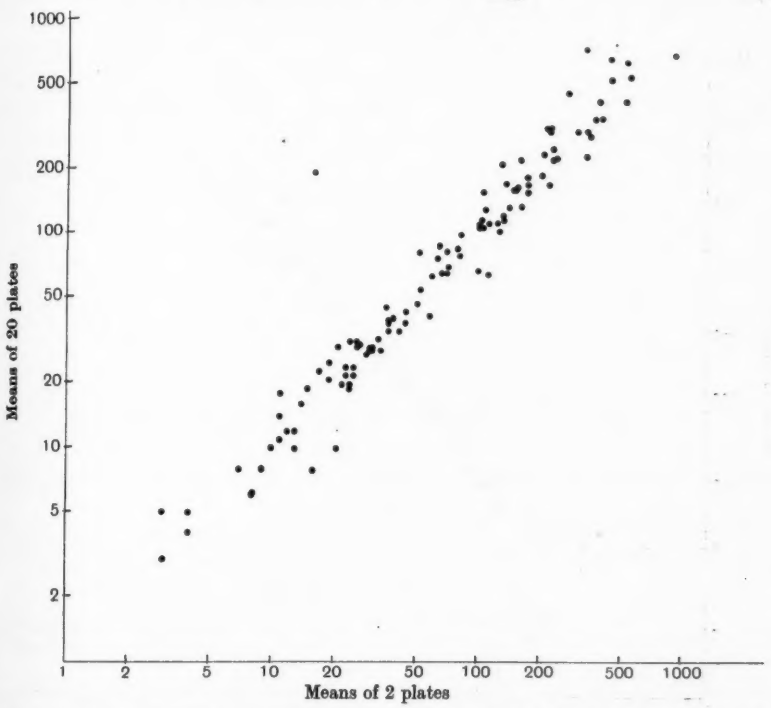

Fig. 1. Correlation diagram showing relation between mean counts of two plates and of 20 plates.

Where M_{20} equals the mean of the 20 plate counts, and M_x equals the mean of the first two, five or ten plate counts respectively. The percentage difference may be either positive or negative according to the value of M_x being less or greater than the value of M_{20}. Table VII gives the frequency of these percentage differences, the frequencies being expressed as percentages of the number of samples, viz. 120. Fifty-one samples with mean counts of less than 50 colonies on the 1/100 dilution plates have been included in the table. In such samples the actual differences between the mean counts obtained from the two, five or ten plates and from the 20 plates are generally very small but, owing to the effect of the low value of the means, the percentage differences

between the mean plate counts are in some instances high. The marked uniformity of the parallel plate counts of such samples is clearly shown by Table VIII which gives the frequencies of the actual differences between the mean colony counts of two, five and ten plates, and of the corresponding 20 plates. In only a very small proportion of the samples is the actual difference between the means greater than 5·0.

Table VII shows that in 36·5 per cent. of the samples, the mean colony counts of the first two plates differ by more than 19·4 per cent. from those

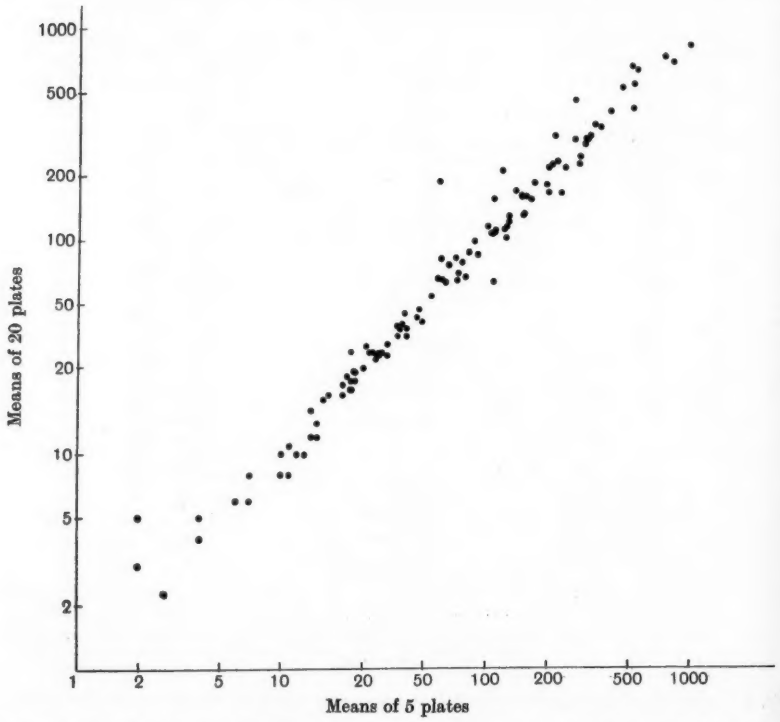

Fig. 2. Correlation diagram showing relation between mean counts of five plates and of 20 plates.

of the corresponding 20 plates. The percentage of samples, however, is reduced to 21·5 per cent. when a deduction of 15 is made as a percentage allowance for samples having mean plate counts of less than 50. In the case of these samples with low mean counts, although the percentage difference between the means of the first two and the 20 plate counts is over 19·4 the actual difference is of no practical importance. If the mean of the 20 plate counts is taken as a standard, it is obvious that in a large proportion of the samples, viz. 21·5 per cent., the mean count of two plates is not satisfactory. For example, in the case of one sample, No. 56, the mean of the two plate counts was 339, and

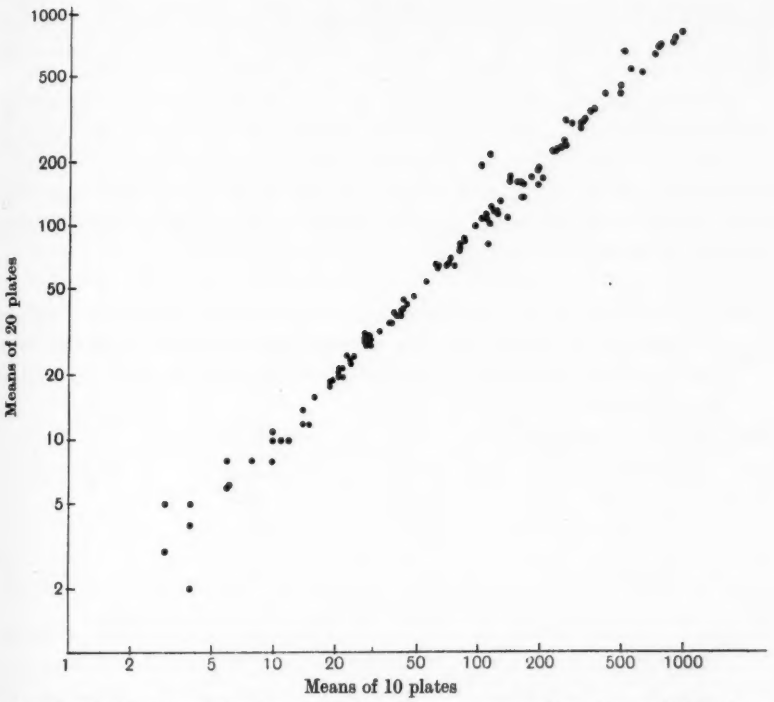

Fig. 3. Correlation diagram showing relation between mean counts of ten plates and of 20 plates.

Table VII. *Comparison of the percentage differences between the means obtained from two, five and ten plates with that obtained from 20 plates, expressed as a frequency table, all samples included.*

Percentage difference of mean count of 2, 5 and 10 plates from that of 20	Samples (%)		
	2 plate series	5 plate series	10 plate series
0 – 4·4	23·3	29·3	52·5
4·5– 9·4	17·5	20·0	24·2
9·5–14·4	12·5	18·3	7·5
14·5–19·4	10·0	13·3	5·8
19·5–24·4	10·0	2·5	2·5
24·5–29·4	5·0	5·0	3·3
29·5–34·4	7·5	4·3	—
34·5–39·4	3·3	2·5	0·8
39·5–44·4	4·2	0·8	0·8
44·5–49·4	0·8	0·8	1·7
49·5–54·4	0·8	0·8	—
54·5–59·4	0·8	0·8	—
59·5–64·4	—	0·8	—
64·5–69·4	—	—	—
69·5–74·4	0·8	0·8	—
74·5–79·4	—	—	—
79·5–84·4	—	—	—
84·5–89·4	—	—	—
89·5–94·4	0·8	—	—
94·5–99·4	—	—	—
Over 99·4	2·5	—	0·8

the mean of the 20 plate counts 240, the percentage difference between the means being 41·25 per cent. Using only two plates, therefore, the bacterial content of this sample would be estimated as 33,900 instead of 24,000.

It is evident from Table VII that the results obtained from the use of five parallel plates are more satisfactory. In nearly 50 per cent. of the samples the difference between the mean count of five plates and that of 20 plates does not exceed 10 per cent. In approximately 19 per cent. of the samples, *i.e.* 23 samples, the difference exceeds 19·4 per cent. These 23 samples may be divided into three groups.

Table VIII. *Comparison of the actual difference between the means obtained from two, five and ten plates with that obtained from* 20 *plates, expressed as a frequency table. Samples giving mean colony counts of less than* 50 *on* 1/100 *dilution plates.*

Actual difference of mean count of 2, 5 and 10 plates from that of 20 plates	Samples (%)		
	2 plate series	5 plate series	10 plate series
0 – 4·4	74·5	90·2	100
4·5– 9·4	19·6	9·8	—
9·5–14·4	3·9	—	—
14·5–19·4	2·0	—	—
Over 19·4	—	—	—

Table IX. *Samples with mean plate counts of less than* 50, *and with a percentage difference between means of five and* 20 *plates of* 19·5 *per cent. or more.*

Sample No.	5 plates mean count	20 plates mean count	Difference between means	Percentage difference
204*x*	3	2	1	50·0
205*x*	2	3	1	33·3
170	4	5	1	20·0
204	2	5	3	60·0
159	10	8	2	25·0
162	11	8	3	37·5
196	13	10	3	30·0
160	15	12	3	25·0
206	22	30	8	26·7

(1) Samples, nine in number, which gave mean plate counts of less than 50 (see Table IX). It will be seen that the actual differences between the means of the five and of the 20 plate counts are of no practical significance. The high values of the percentage differences are due to the low values of the means.

(2) Samples, four in number, with very high mean plate counts (see Table X). Owing to the large number of colonies on the plates, only an approximate count could be made, and this may be partly responsible for the large differences between the mean counts.

(3) Samples, ten in number, giving irregular plate counts (see Table XI). In only five of these samples, viz. Nos. 218, 14, 66, 198 and 23 are the differences between the mean counts so great as to give highly misleading results where five plates are used.

With the exception, therefore, of about 4 per cent. of the samples, of which the plate counts are highly irregular, reliance can be placed on the mean counts obtained from the five 1/100 dilution plates, provided that the bacterial content of the milk does not exceed 50,000 or 60,000 organisms per c.c., *i.e.* that the number of colonies on the plates does not exceed 500 or 600.

Table VII shows that there is a very close agreement between the mean counts of ten and 20 parallel plates. In only 10 per cent. of the samples does the difference between the mean counts exceed 19·4 per cent., and if allowance is made for the samples with mean plate counts below 50, then only 4 per cent. exceed this difference. It should be noted that where the mean plate count

Table X. *Samples with high mean plate counts with a percentage difference between means of five and 20 plates of 19·5 per cent. or more.*

Sample No.	5 plates mean count	20 plates mean count	Difference between means	Percentage difference
183*x*	519	698	179	25·6
197*x*	1031	751	280	37·3
196*x*	1120	815	305	37·4
202	2045	1628	417	25·6

Table XI. *Samples with irregular plate counts and with a percentage difference between means of five and 20 plates of 19·5 per cent. or more.*

Sample No.	5 plates mean count	20 plates mean count	Difference between means	Percentage difference
218	105	66	39	59·1
145	60	84	24	28·6
63	110	161	51	31·7
157	233	175	58	33·1
16	140	178	38	21·4
14	59	198	139	70·2
66	219	326	107	32·8
64	534	437	97	22·2
198	271	475	204	42·9
23	120	221	101	45·7

is not above 50, a very close agreement exists between the means obtained from ten and from 20 parallel plates, the actual difference between the means in all instances being less than 4·5.

It is apparent that the results obtained from ten plate counts are more trustworthy than those obtained from two or five. The ten plate method, however, suffers from the disadvantage that the requirements in media and apparatus are high and, apart from the question of cost, the method is consequently not suitable for the routine examination of milk. Further, it has been shown that the five plate method yields results which appear to be sufficiently reliable for this purpose.

SUGGESTED PROCEDURE FOR THE MORE ACCURATE ESTIMATION OF THE BACTERIAL CONTENT OF MILK.

In the routine examination of milk for bacterial content, the following method is recommended. Omit the 1/10 dilution bottle and 1/10 dilution plates, and prepare the 1/100 and 1/1000 dilution plates by the modified methods previously discussed. Use five parallel plates for the 1/100 dilution and two plates for the 1/1000 dilution.

As has already been shown, in samples whose counts amount to as much as 50,000 or more organisms per c.c. the above method yields reliable results. When the bacterial content exceeds 50,000 organisms per c.c., *e.g.* up to 100,000, a reasonable degree of reliance can be placed on the results obtained, owing to the large number of 1/100 dilution plates, which can be used to some extent to confirm the results of the two 1/1000 dilution plates. But if it is desired to obtain fairly accurate estimates when the counts are in the neighbourhood of 100,000 organisms per c.c. at least five plates must be used for the 1/1000 dilution.

Nevertheless the possibility of the occurrence of a serious error is not completely eliminated, even where five plates are used, for in 4 per cent. of the samples the estimates may be misleading. Therefore in grading milk by this method, where the bacterial count exceeds the count which is the limiting standard for the grade, the results should be confirmed by means of a second test upon another sample. While this is advisable where five plates are poured for each dilution, it is essential where only one or two plates are poured, otherwise a milk supply may be condemned unjustly. It must, in fact, always be borne in mind that the most reliable information in regard to the degree of purity of any given milk supply is obtained not from single tests, but from a series of tests performed at intervals over a period of several months, or preferably over a full year.

(*Note.* Since the preparation of this paper my attention has been drawn to the results of recent investigations, published in the present issue of the *Journal* (7), which demonstrate the inadequacy of the present standard agar as a medium for plate counts, particularly for the higher dilutions of milk. While this fact will not invalidate the main conclusions of the present paper, it should be taken into account in any application of the conclusions to the existing "official" method of milk testing. (J. F. M.))

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(*MS. received for publication July* 7, 1932.)

576.809.33:637.12

52. THE BACTERIOLOGICAL EXAMINATION OF MILK

I. MODIFICATION OF THE AGAR PLATE-COUNT METHOD.

II. THE USE OF THE METHYLENE BLUE REDUCTASE TEST AT 15·5° C. AS A METHOD OF DETERMINING THE KEEPING QUALITY OF MILK.

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(With 6 Charts.)

PART I.

For a number of years evidence has steadily been accumulating that the standard agar[1] which is commonly used for the estimation of the numbers of viable bacteria in milk does not always give unimpeachable results. Well-founded criticism of the plate count of milk must be seriously considered, since this test is of fundamental importance in the English system of grading milk and in many schemes for the payment for milk on the basis of quality. Extensive experience with low-count milk has shown that, as a general rule, there is a reasonably good agreement between the counts for the various dilutions of such milk, but for ordinary commercial raw milk there is often a discrepancy between the plate counts of the 1/100 c.c. and 1/1000 c.c. dilutions. For pasteurised milk this discrepancy is even more frequent and more marked. Typical examples are given in Table I.

Table I. *Plate count on standard agar.*

Raw milk		Pasteurised milk	
1/100 dilution	1/1000 dilution	1/100 dilution	1/1000 dilution
420	17	1250	30
1620	67	800	2
1524	84	1348	80
1248	81	232	9
1564	47	1064	38
1600	51	405	14
1628	31	1056	20
1162	67	800	25
448	7	510	0
512	19	2120	70

N.B. The larger figures in these columns are approximations only.

It has been observed that these discrepancies are very frequently associated with the presence on the 1/100 c.c. plate of large numbers of minute colonies which are rare or entirely absent on the 1/1000 c.c. plate. This is

[1] Of the composition recommended by the Ministry of Health.

suggestive of a deficiency in the nutritive properties and perhaps in the buffer value of the standard agar which is therefore unable to support the full growth of some types of bacteria. When such bacteria are present in milk they begin to develop on the 1/100 c.c. plate, presumably because a small quantity of additional nutriment is supplied by the 1/100 c.c. of milk added with the dilution, but the growth is restricted to the formation of minute colonies, the enumerations of which are always inaccurate. On the 1/1000 c.c. plate the extra nutriment added as milk with the dilution is so small as to be ineffective in starting the growth of these bacteria, or at any rate in allowing them to grow into visible colonies.

If this explanation be true it would imply that these discrepancies would not arise when dealing with clean milk of a low bacterial content, since the dilutions that would be plated (1/10 c.c. and 1/100 c.c.) would supply sufficient milk to counterbalance, at any rate in the lower dilution, any nutritional deficiencies existing in the standard agar. In practice, the agreement between dilutions has usually been close enough to warrant the assumption that this is in fact the case. The serious discrepancies have only been observed for pasteurised milk and for some types of commercial raw milk.

The media now in use in England for the examination of milk samples were designed for use with raw milk of a low bacterial content, and it was not anticipated at the time that the standard agar, which fulfils the necessary conditions of cheapness and ease of preparation, would prove to be seriously deficient for some types of milk. Unfortunately, anomalies similar to those recorded above have been frequently observed, and the fact that the standard media are not satisfactory in all circumstances has led to such severe criticism of the official methods of plate counting that a detailed re-investigation of the whole question is at present being undertaken at the London School of Hygiene and Tropical Medicine.

One criticism that has frequently been made is that the standard agar contains no sugar and therefore cannot be favourable to the growth of some types of bacteria commonly found in milk, notably the lactic acid bacteria. Casein agar (prepared from peptic digest of casein, and containing dextrose)(1) has often been recommended as a medium which might with advantage be substituted for standard agar. Comparative tests, using raw milk of the type given in Table I, failed to demonstrate the superiority of casein agar. Rather, they indicated that standard agar gave better results for this particular purpose.

Experience suggested that standard agar could be converted into a more suitable medium for general plate counting by the addition of small quantities of milk at the time of plating. In practice there is no difficulty in this procedure except that it is rather inconvenient. No difficulty has been encountered because of opacity of or precipitates in the medium.

It is, however, clearly essential that any medium in general use for the purposes stated above shall be as uniform and reproducible as possible, and it might be urged in this connection that the composition of milk would vary

from laboratory to laboratory. In view, however, of the great uniformity in composition of milk in respect of all the major constituents except fat (2), if the milk used for the enrichment of standard agar were always drawn from a large bulk the difficulty would largely, if not completely, disappear. Experiments are in progress which promise to permit the addition of milk, or its essential components, at the time of preparation of the agar.*

The method of making dilutions in 5 per cent. milk instead of in water has also been found to be quite successful, but if accurately measured quantities are stored, adequate controls of sterility are essential. It has also been suggested that the addition of other substances of definite chemical composition and obtainable in a pure state might give equally good results. The results of experiments bearing on this problem are presented in this paper.

Series I.

This series of tests was designed chiefly to explore the possibility of the reinforcement of standard agar by the addition of various substances. For this purpose the sugars lactose, dextrose, and galactose, were chosen because of their presence in, or association with, milk, and because they are readily obtainable in a pure state. For purposes of further comparison, autolysed yeast extract, which has been shown to be very favourable for the growth of many types of lactic acid bacteria (3), (4), and milk, both raw and boiled for 30 min., were also used.

A single batch of standard agar formed the basic medium throughout the experiment. The sugars, in the proportions lactose 0·5 per cent., dextrose 0·5 per cent., galactose 0·5 per cent., and dextrose 0·25 per cent. + galactose 0·25 per cent., were added to the agar just before tubing and sterilising. The yeast extract (1/10 c.c.) and the milk (1/10 c.c.) were added at the time of plating. This quantity of milk was chosen because the preliminary observations had indicated that the quantity (1/100 c.c.), added with 1 c.c. of the 1/100 c.c. dilution in the ordinary course of plating, was not always sufficient to induce the growth of those bacteria for which standard agar alone was unsatisfactory. It was thought advisable to add a quantity that was well above this minimum.

The number of dilutions was varied according to the suspected quality of the sample of milk under test, the aim being to obtain at least two countable plates for comparison. Each sample of milk was shaken 25 times with an excursion of 1 ft. before withdrawing the required quantity with the pipette. Blow-out 1 c.c. pipettes plugged with cotton-wool were used. The dilutions were made in 9 c.c. quantities of normal saline, and mixing secured by blowing into the mixture and by raising and lowering in the pipette six times in all cases. The eight plates from each dilution were made at the same time, so

* Whilst this paper was in the press a paper by Demont and Dorner (*Landw. Jahrb. Schweiz*, 1931) was received. These authors secured an increase in the plate count by the use of whey and a special peptone.

that they were, as far as possible, comparable with one another. Care was taken to ensure that the volume of agar used in plating was the same in all cases, so that the depth of medium in the plates of standard size (10 cm. diam.) was always the same. The counts were carefully made after incubation of the plates at 37° C. for 48 hours. The size of the colonies and the ease of counting were noted as well as the actual numbers. Throughout the experiment the milk added to the agar was from the same cow, and was taken

Table II. *Plate count per* 1 *c.c.*

Samples of milk	Standard agar	Standard agar + lactose	Standard agar + dextrose	Standard agar + galactose	Standard agar + dextrose and galactose	Standard agar + yeast extract	Standard agar + milk (raw)	Standard agar + milk (boiled)
Pasteurised:								
(1)	146,000	175,000	147,000	136,000	157,000	81,000	227,000	179,000
	1,140,000	1,070,000	1,060,000	750,000	420,000	1,090,000	1,150,000	1,030,000
	5,560,000	5,180,000	4,780,000	3,540,000	3,770,000	6,130,000	9,180,000	9,400,000
	68,000	42,000	85,000	66,000	59,000	68,000	133,000	139,000
	80,000	110,000	70,000	120,000	70,000	70,000	420,000	400,000
	214,000	277,000	268,000	299,000	320,000	315,000	298,000	287,000
	132,000	101,000	139,000	192,000	122,000	137,000	163,000	190,000
	16,200	16,200	18,500	18,000	16,800	24,100	23,700	23,200
	9,600,000	16,800,000	11,300,000	12,200,000	10,200,000	11,800,000	21,400,000	18,300,000
(2)	3,400	3,900	6,700	9,000	5,000	7,900	6,500	10,800
	44,000	147,000	108,000	152,000	112,000	161,000	165,000	143,000
	11,800	16,500	17,400	16,200	15,700	13,800	19,300	23,500
	55,000	80,000	69,000	89,000	106,000	114,000	75,000	123,000
	38,000	63,000	32,000	45,000	71,000	52,000	110,000	91,000
	8,900	12,700	14,600	14,900	11,400	17,500	26,500	21,800
	15,000	93,000	71,000	51,000	92,000	17,000	122,000	108,000
(3)	450,000	500,000	523,000	373,000	624,000	343,000	973,000	730,000
	175,000	417,000	331,000	239,000	295,000	114,000	808,000	790,000
	43,300	52,600	—	—	—	68,000	76,500	98,000
Raw commercial:								
(1)	760,000	760,000	660,000	720,000	700,000	850,000	1,050,000	1,040,000
	30,000	32,000	36,000	32,000	41,000	41,000	46,000	39,000
(2)	1,300,000	1,690,000	1,050,000	1,210,000	1,420,000	1,790,000	2,660,000	1,820,000
	5,200,000	6,850,000	5,360,000	5,270,000	5,480,000	6,100,000	5,820,000	6,540,000
(3)	2,110,000	3,730,000	2,790,000	1,800,000	2,780,000	3,950,000	5,220,000	4,380,000
	2,870,000	3,630,000	3,350,000	3,800,000	3,060,000	4,170,000	4,290,000	4,070,000
(4)	3,090,000	2,970,000	2,860,000	2,870,000	2,730,000	2,870,000	6,500,000	3,390,000
(5)	1,040	700	700	1,200	1,500	1,700	1,400	1,200
	850	580	690	680	640	750	600	1,000
Raw (clean):								
	*30,000	—	—	—	—	—	—	41,000
	27,000	—	—	—	—	—	—	20,000
	4,150	—	—	—	—	—	—	4,300
	4,050	—	—	—	—	—	—	4,600
	370,000	—	—	—	—	—	—	410,000
	21,000	—	—	—	—	—	—	21,000
	20,000	—	—	—	—	—	—	22,000
	19,000	—	—	—	—	—	—	22,000

* Means of duplicate plates.

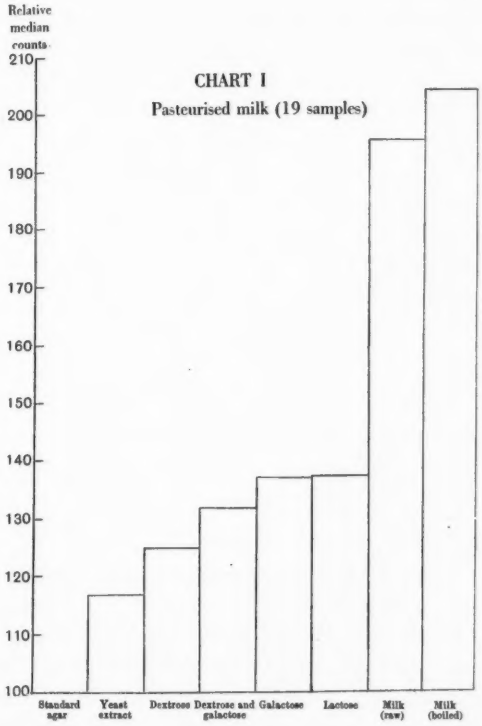
direct from the milking pail into a sterile bottle and kept in an ice chest until used a few hours after milking. Control plates were made of the boiled and unboiled milk so that any necessary adjustments could be made in the counts for the milk samples. With two exceptions these adjustments were very small even for the unboiled milk.

Various types of milk were tested, including pasteurised milk from three sources, commercial milk from five farms, 28 samples in all. The actual plate

counts are given in Table II, which illustrates the wide variations in the quality of the samples tested and the extent to which the plate count of each sample was influenced by the addition of the various substances.

In very few cases could the counts of both 1/100 c.c. and 1/1000 c.c. dilutions be determined. There was thus little opportunity for further observation of the correction of the discrepancies noted earlier in this paper.

The relative values of the various additions to the standard agar are illustrated in Charts I and II, in which the medians of the counts on the various media have been plotted. (For purposes of comparison the count on standard



agar has, in every instance, been reduced to 100, and the counts on the other media calculated on this basis.) The greater general response of the flora of pasteurised milk to the stimulus of the various added substances is clearly indicated, the addition of the various sugars being without significant influence on the plate count of raw milk. For both raw and pasteurised milk the highest counts were obtained by the addition of milk, but it is notable that whilst the favourable influence of yeast extract is nearly as great as that of milk for the varied flora of raw milk, its influence is usually negligible for the more specialised flora of pasteurised milk.

Since the addition of the sugars appeared to have little real influence on the plate count of either raw or pasteurised milk many of the results have been omitted from Chart III for the sake of clarity, and only three curves have been plotted, viz. the relative counts (standard agar being taken as 100) on standard agar, on standard agar + lactose, and on standard agar + boiled milk.

It appears that the addition of lactose to the standard agar had only a comparatively slight influence on the plate count of either raw or pasteurised milk used in these experiments. In very few cases could it be regarded as

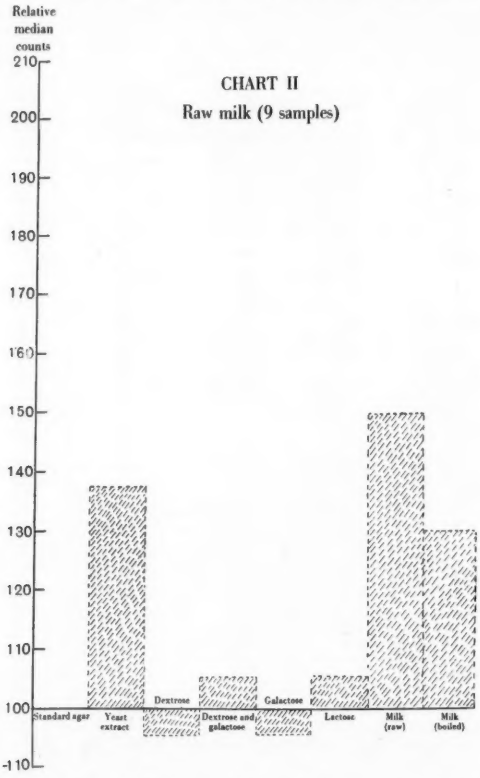


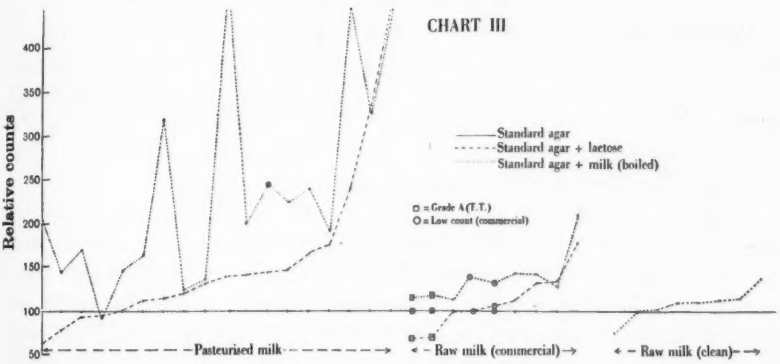
CHART II

Raw milk (9 samples)

having any real value. The addition of milk, however, often had a marked influence on the plate count. For pasteurised milk there was almost invariably an increase, rarely less than 30 per cent., frequently more than 100 per cent. For raw milk, the addition of milk to the standard agar had no apparent significance. The increase in count rarely exceeded the limits of error of sampling, etc. (say, ± 30 per cent.). This was true for the samples of commercial milk, many of which had high plate counts, but it was even more evident for the samples of low count milk, the figures for which have been appended to those of the original series. (Table II.)

The favourable influence of the addition of milk was not always greatest for those samples having a high plate count. It was presumably more closely connected with the types of bacteria than with the actual count, for it was very frequently associated with the presence of minute colonies on the standard agar plates. Unfortunately, only one of the samples of pasteurised milk produced true pin-point colonies, but it is significant that in this case the addition of milk increased the count by about 350 per cent., and entirely eliminated the pin-point colonies.

The addition of milk tended to increase the size and density of the colonies, thus making for greater ease in counting, an important point in securing an accurate count. The addition of yeast extract had an even greater influence in this direction, but the selective action of this substance and its variable composition rule it out from serious consideration as a means of reinforcement of standard agar for the determination of the plate count of milk.



Series II.

Using the same technique as in Series I, except that dilutions were made with 10 c.c. milk and 90 c.c. saline, a second series of tests was carried out on officially designated pasteurised milk from two sources during 1929 and 1930. This series was concerned with the comparative study of the plate counts of the 1/1000 c.c. dilutions on standard agar, on standard agar + lactose (0·1 per cent.) and on standard agar + boiled milk (1/100 c.c.). This quantity of milk was chosen because it is equal to that which is added with the 1/100 c.c. dilution and which appeared to be sufficient to correct the nutritive deficiencies of standard agar in many cases. The concentration of lactose (0·1 per cent.) was chosen because, although small, it was still double the amount added with the above quantity of milk, and enough to indicate the effect, if any, of the lactose added with the milk. It was expected that these additions would eliminate the discrepancies between the plate counts of the 1/100 c.c. and 1/1000 c.c. dilutions so frequently found on standard agar, and so lead to correlation between the counts. This has proved to some extent to be the

case, as is shown by the figures given in Table III. The addition of milk gave better results than the addition of lactose; in several cases the latter counts were lower than the corresponding counts on standard agar.

Table III. *Pasteurised milk. Plate counts of pasteurised milk.*

Standard agar		Standard agar + lactose	Standard agar + milk
1/100 c.c.	1/1000 c.c.	1/1000 c.c.	1/1000 c.c.
1250	30	31	380
800	2	34	416
1348	80	40	100
232	9	7	58
1064	38	116	76
405	14	37	67
1056	20	110	120
800	25	50	185
510	0	20	77
2120	70	205	235

N.B. The larger figures are approximations only.

Although such discrepancies were frequent they did not invariably occur. There were usually, however, considerable differences between the counts on the three media under comparison, and generally the count on standard agar + milk was much higher than either of the others, thus confirming the results of the first series. The larger number (233) of samples tested in this series has allowed of somewhat different methods of analysis.

Although the count on standard agar + milk was usually the highest, it was sometimes lower than that on standard agar + lactose, and on occasions even lower than on standard agar (Table IV), but these differences were then usually quite small and within the assumed range of error. The count on standard agar + lactose was, on the other hand, frequently lower than that on standard agar, and sometimes large differences were found. It would appear that lactose, either directly or indirectly as lactic acid, exerts an inhibitory action on the growth of certain types of bacteria. In general the addition of lactose acts favourably on the growth of streptococci but unfavourably on that of spore formers and thermophilic types, but sufficient data are not yet available to warrant further discussion on this point.

Table IV.

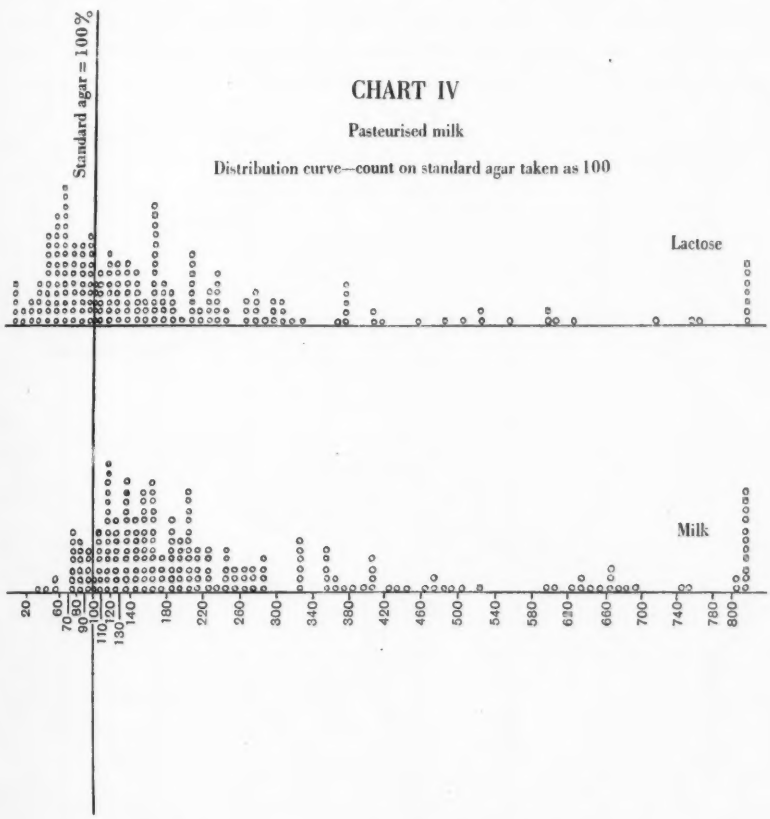
Total No. of samples tested	No. of samples for which the count on standard agar + lactose was lower than the count on		No. of samples for which the count on standard agar + milk was lower than the count on	
	Standard agar	Standard agar + milk	Standard agar	Standard agar + lactose
233	81	157	23	68

The figures have been subjected to other methods of analysis in order to study further the relative merits of the three types of media when used for the determination of the bacterial count of pasteurised milk.

In Chart IV is shown the frequency distribution curve of the results obtained by expressing the counts on standard agar + milk, and standard

agar + lactose as percentages of the count on standard agar, this last being taken as 100 in each case. The percentages are grouped in tens, *e.g.* 101–110, 221–230, etc.

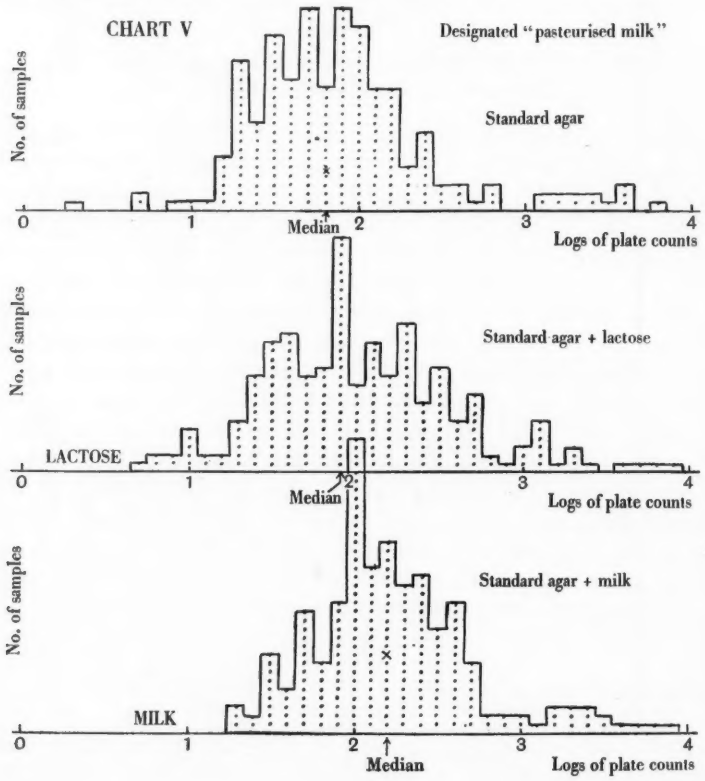
It will be seen that, if the very high figures (*e.g.* over 250) are ignored in each case, the bulk of the figures for the standard agar + lactose are distributed almost equally on both sides of the 100 per cent. line, *i.e.* the position



of equality with the figures for standard agar, whilst the majority of the figures for the standard agar + milk are definitely to the right of this position. Of the figures for the standard agar + lactose, 45 have exceeded the 250 per cent. zone, but 71 of the standard agar + milk figures have exceeded this limit. The median for standard agar + lactose is 140–150 per cent., whereas for standard agar + milk it is 200–210 per cent.

In this method of expressing the counts as percentages of the count on standard agar, the unit of comparison is admittedly arbitrary, and, as this paper shows, frequently unreliable. In Chart V, therefore, a frequency curve

has again been plotted, but the logarithms of the actual plate counts have been used. The shift to the right of the main bulk of figures is not so marked as in Chart IV, although it is still evident. The most significant feature of these curves is the greater compactness of the logarithms of the counts for standard agar + milk, none being less than 1·3, whereas for the standard agar the lowest is 0·3 and for the standard agar + lactose 0·7. The curve for the standard agar + milk rises more steeply than either of the others, indicating that the lower values on standard agar and on standard agar + lactose are due to the inadequacy of the medium, since they represent the comparative counts ot samples of milk on each of the three media.



SERIES III.

Effect of time and temperature of incubation.

During the course of these experiments it was often found that the plates were very difficult to count when the counting was made immediately after 48 hours' incubation at 37° C. according to the general practice. No particular difficulty was reported for low-count raw milk, and only rarely for

ordinary commercial raw milk, but for pasteurised milk the minute size and transparency of the colonies frequently made the work very troublesome and therefore inaccurate. It was noticed, however, that if such plates were left on the laboratory bench overnight the difficulty largely disappeared and the plates could be counted with comparative ease.

The method of counting the plates at the end of 48 hours' incubation at

Table V. *Plate counts and conditions of incubation.*

	1/1000 c.c. dilution on standard agar				1/1000 c.c. dilution on standard agar + milk				
Date	48 hr. at 37° C.	66 hr. at 37° C.	66 hr. at 37° C., 48 hr. at 22° C.	66 hr. at 37° C., 48 hr. at 22° C., 14 days at room temp.	48 hr. at 37° C.	66 hr. at 37° C.	66 hr. at 37° C., 48 hr. at 22° C.	66 hr. at 37° C., 48 hr. at 22° C., 14 days at room temp.	Quantity of milk added to each plate c.c.
24. ii. 32	25	100	102	—	80	126	130	—	
	27	63	72	—	32	53	60	—	
	18	41	41	—	120	132	142	—	
	16	50	46	—	110	106	108	—	
	3	9	20	—	25	41	40	—	
16. iii. 32	5	18	56	56	18	36	76	76	
	5	22	30	28	4	8	25	25	
31. iii. 32	130	160	184	—	190	165	256	—	
	24	33	46	50	45	48	70	53	
	34	48	72	68	41	64	60	64	1/100
	9	27	45	53	46	54	56	58	
	4	10	20	22	20	30	38	40	
	12	15	16	22	15	19	20	26	
13. iv. 32	30	30	45	59	45	68	85	74	
	18	20	37	41	42	75	75	68	
	27	45	68	62	36	53	80	53	
	12	29	33	32	68	60	68	60	
	14	50	45	44	12	38	38	52	
	13	38	53	61	24	40	44	38	
	23	44	45	48	27	42	52	46	
26. v. 32	62	160	130	—	262	254	340	—	
	33	68	79	—	45	80	91	—	
	150	400	366	—	360	520	510	—	
	20	85	79	—	60	102	121	—	
	15	40	39	—	170	180	182	—	
9. vi. 32	34	46	63	—	430	330	530	—	
	29	80	117	—	480	600	600	—	
	17	17	28	—	400	432	900	—	
	14	27	44	—	19	80	57	—	
	10	36	46	—	30	52	53	—	
	13	62	72	—	47	69	47	—	1/10
22. vi. 32	90	96	112	—	520	560	600	—	
	10	36	43	—	156	200	280	—	
	8	30	40	—	10	20	28	—	
	26	86	50	—	280	300	260	—	
6. vii. 32	4	33	45	—	1120	1140	1400	—	
	6	30	69	—	43	51	82	—	
	2	16	37	—	104	132	160	—	
	4	30	37	—	1240	1500	1400	—	
	13	40	86	—	75	103	134	—	

37° C. has often been criticised because of the shortness of this period, and of the unsuitability of this temperature for the growth of many types of bacteria, but it has been retained for reasons of economy of time and incubator space. When a number of trial plates were counted at the end of 48 hours' incubation at 37° C. and recounted after holding for a further 48 hours at 22° C. there was a considerable increase in the number of colonies recorded at the second counting.

A third series of tests was then instituted in which a comparison was made of the counts of pasteurised milk on the plates of 1/1000 c.c. dilution after different periods of incubation. For purposes of further comparison the milk was plated on standard agar and on standard agar + milk. At first 1/100 c.c. of sterile milk was added to each plate, but later this quantity was increased to 1/10 c.c. Each plate was counted three times, viz. after

Table VI. *Percentage increase in plate counts with increased incubation.*

	Standard agar			Standard agar + milk			Quantity of milk added to each plate
Date	48 hr. at 37° C.	66 hr. at 37° C.	66 hr. at 37° C., 48 hr. at 22° C.	48 hr. at 37° C.	66 hr. at 37° C.	66 hr. at 37° C., 48 hr. at 22° C.	c.c.
24. ii. 32	100	400	408	100	157·5	162·5	
	100	233·3	266·7	100	165·6	187·5	
	100	227·8	227·8	100	110	118·3	
	100	312·5	287·5	100	96·4	98·2	
	100	300	666·7	100	164	160	
16. iii. 32	100	360	1120	100	200	422·2	
	100	440	600	100	200	625	
31. iii. 32	100	123·1	141·6	100	86·4	134·7	
	100	137·5	191·7	100	106·7	155·6	
	100	141·1	211·7	100	156·1	146·3	1/100
	100	300	500	100	117·4	120·3	
	100	250	500	100	150	190	
	100	125	133·3	100	126·7	133·3	
13. iv. 32	100	100	150	100	151·1	188·9	
	100	111·1	205·6	100	178·6	178·6	
	100	166·7	251·7	100	147·2	222·2	
	100	241·7	275	100	88·2	100	
	100	357·1	321·4	100	316·7	316·7	
	100	293·3	407·8	100	166·6	183·3	
	100	191·4	195·6	100	155·6	192·6	
26. v. 32	100	258	209·6	100	96·9	129·8	
	100	206·1	239·4	100	177·8	202·2	
	100	266·7	244	100	144·4	141·8	
	100	425·5	395·0	100	170	201·7	
	100	266·7	260	100	105·9	106	
9. vi. 32	100	135·3	185·2	100	76·7	123·2	
	100	275·8	403·4	100	125	125	
	100	100	164·7	100	108	225	
	100	192·9	314·4	100	421	300	
	100	360	460	100	173·3	177·8	1/10
	100	488·1	553·9	100	146·8	100	
22. vi. 32	100	106·4	124·4	100	107·6	115·4	
	100	360	430	100	128·2	179·5	
	100	375	500	100	200	280	
	100	330·8	192·3	100	107·1	92·8	
6. vii. 32	100	825	1125	100	128·6	125·0	
	100	500	1150	100	118·6	190·6	
	100	800	1850	100	127	153·6	
	100	750	925	100	121	112·9	
	100	307·7	661·6	100	137·3	178·6	

incubation at 37° C. for 48 hours, after further incubation for 18 hours, *i.e.* 66 hours, at 37° C., and after an additional incubation of 48 hours at 22° C. A few plates were counted for the fourth time after being allowed to stand at room temperature for 14 days. A lens of 4*x* magnification and a specially designed counting chamber of standard illumination were always used for the

counting, the other apparatus and technique being those used in Series II. Typical figures are given in Table V.

There was, almost without exception, a definite increase in the number of colonies counted when the period of incubation at 37° C. was extended to 66 hours, and usually a further increase after the additional 48 hours' incubation at 22° C. This treatment appeared to allow sufficient time for the development of all the colonies that will grow under the conditions of the experiment, since there was rarely any appreciable change in the count during subsequent storage at room temperatures for 14 days.

A very important point which cannot be indicated in the table is the fact that not only was there an increase in the number of colonies during the extra 18 hours' incubation at 37° C., but there was also a marked improvement in their visibility. The strain of counting was much lessened thereby.

The increase in the number of colonies as a result of prolonging the incubation period was observed whether the platings were made on standard agar or on standard agar + milk, but the increase was less marked on the latter medium. This is evident from Table VI, in which the figures given in Table V have been recalculated on another basis, taking the first count (after 48 hours' incubation at 37° C.) as 100 in every case. For instance, of the 40 platings recorded 12 showed an increase of less than 100 per cent. on standard agar when the incubation period was extended to 66 hours as compared with 35 on standard agar + milk. The least change in count was observed when the milk was plated on standard agar to which 1/10 c.c. of milk was added at the time of plating. This is further evidence that the addition of milk converts the standard agar into a more satisfactory medium, since not only does it result in a higher total plate count, but the value obtained even at the end of the comparatively short incubation period now employed is nearer its maximum than is the value obtained with standard agar. The error due to the short period of incubation is less than is the case when standard agar is used.

Discussion.

The plate count of milk has been recognised as being of prime importance in the determination of the hygienic quality of milk, and it is essential therefore that its results shall be sufficiently representative to give a reliable judgment, although of necessity the figures cannot be absolute. The keeping quality test is also widely used in judging milk. It is obvious that milk having a low plate count will have a greater keeping quality than milk having a high, and in general the rule holds good that the lower the plate count the greater the keeping quality of a sample of milk. The correlation between the two tests is not, however, always as close as is desirable, and this is not surprising when their difference in scope is considered. It is possible that, as the methods of plate counting are modified so that a more truly representative idea of the bacterial content of the milk is obtained, a closer agreement

between the tests will be possible. As a rule, the method which gives the highest count whilst still allowing the test to be simple and inexpensive, may be expected to prove the most satisfactory for the general routine examination of milk.

The evidence that has been brought forward in this paper all tends towards the same conclusion, that the addition of milk to standard agar would convert it into a medium adequate for many purposes for which, in its present form, it is unreliable, whilst a slightly prolonged period of incubation would give counts which would more nearly represent the true count on the medium. These modifications would in many cases lead to higher plate counts.

It was pointed out earlier in this paper that when milk of a low bacterial content is plated on standard agar, the possibility of using low dilutions corrects to some extent the inadequacy of the medium, since, for example, when 1/10 c.c. of milk is added to each plate with the inoculum the count is not appreciably affected by the addition of further quantities of milk to the medium. For milk of high bacterial content, on the other hand, the amount of milk added with the inoculum is usually too small to be effective and the plate counts would frequently be too low and misleading. Milk of a poor quality would thus often be judged too leniently and high-grade milk would suffer in comparison if standard agar were used for the plating process. This erroneous judgment would occur even more frequently in the case of pasteurised milk which has a very specialised flora, all the more so because the plate of the 1/1000 c.c. dilution must frequently be counted. This will influence the number of samples which conform to the standards of grading when the standards are expressed in terms of the plate count per 1 c.c.

This is clearly illustrated in Table VII, in which the results of tests on pasteurised milk carried out at fortnightly intervals over a period of 2 years have been summarised. There is a very significant difference between the

Table VII. *Number of samples of milk which failed to reach the standard of a plate count of not more than* 100,000 *per c.c.*

	Plated on standard agar		Plated on standard agar + milk		
Total No. of samples	Incubated 48 hr. at 37° C.	Incubated 66 hr. at 37° C.	Incubated 48 hr. at 37° C.	Incubated 66 hr. at 37° C.	Quantity of sterile milk added to each plate c.c.
233	81	—	155	—	1/100
84	2	2	7	8	1/100
56	5	5	23	26	1/10

results obtained when (*a*) the standard method of plate counting and (*b*) the modifications discussed in this paper were used. The difference was accentuated during the warmer months when the counts of a high proportion of the samples were near the border line.

Figures are not available for a comparison of the extent to which these modifications of the standard plate count method would affect the grading

of raw milk, but the general effect is seen in Table II. They would probably be of similar general order, but less significant.

The introduction of the modified methods would, on the whole, have the effect of tightening up the grading of milk. This would be expected to be correlated with a general improvement in the quality of the milk placed in the various grades, that is to say, the average keeping quality of milk having a given range of plate count is greater when the method employed gives the highest count. This is illustrated in Table VIII, in which the mean counts and mean keeping qualities for various arbitrary groups are compared when standard agar and standard agar + milk were taken as the plating media. The substitution of standard agar + milk for standard agar relegates some of the poorer samples of each group to a lower grade, and thus the average quality of each group is improved, and the standard of the whole is raised.

The occurrence of a few abnormal counts may seriously affect the mean. Hence the median counts and keeping qualities for each group have been compared in Table IX. The results were substantially the same.

Table VIII.

Groups according to plate count per 1 c.c.	Standard agar as plating medium		Standard agar + milk as plating medium	
	Mean count	Mean keeping quality hr.	Mean count	Mean keeping quality hr.
Not more than 30,000	21,700	52·1	23,800	54
30,001–100,000	63,200	52·3	72,000	53·5
100,001–200,000	141,000	44·8	144,000	49·0
200,001–500,000	289,000	38·2	320,000	46·4
More than 500,000	2,680,000	28·5	3,250,000	39·2

N.B. Counts of more than 10,000,000 have been excluded.

Table IX.

Groups according to plate count per 1 c.c.	Standard agar as plating medium		Standard agar + milk as plating medium	
	Median count	Median keeping quality hr.	Median count	Median keeping quality hr.
Not more than 30,000	21,000	54	27,000	57
30,001–100,000	62,000	54	65,000	54
100,001–200,000	137,000	42	140,000	48
200,001–500,000	272,000	36	320,000	42
More than 500,000	2,370,000	30	2,000,000	30

Another point which arises is the fact that tests for the sterility of milk vessels and plant may often be misleading when standard agar is used as the plating medium. The washings of these vessels may contain bacteria which grow well in milk but not on standard agar, and therefore are left out of consideration in an ordinary plate count, although their presence might appreciably affect the quality of milk placed in the vessels. The addition of a small quantity of milk to the standard agar allows these bacteria to grow, and would therefore give a better idea of the true extent of contamination of the vessels.

SUMMARY.

It has been shown that neither lactose, nor other sugars tested, casein peptone, nor yeast extract can satisfactorily replace milk as a means of correcting discrepancies which are frequently observed between the plate counts of the 1/100 c.c. and 1/1000 c.c. dilutions of milk on standard agar. Experiments are in progress by which it is hoped that the objections to the addition of milk to standard agar either at the time of making or at the time of plating will be removed, and a medium be evolved which will combine the ease of making and the transparency of standard agar with the efficiency of the medium enriched with milk.

PART II.

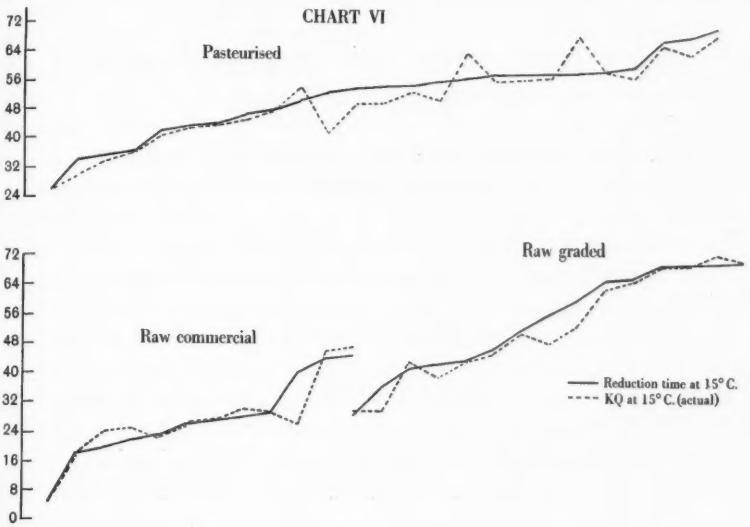
The keeping quality test as at present carried out(5) is determined by observation of the development of taint in the milk. The accuracy of this test depends, therefore, on the delicacy of the taste of the observer. Since the sense of taste is not equally developed in all individuals the results are not always comparable.

During a study of the reductase test at various temperatures lower than 40° C. it was found that a very close agreement existed between the keeping quality of samples of milk, as determined by frequent tasting and their reduction time at 15·5° C. This is no doubt due to the fact that both the keeping quality and the reduction time depend to a very large extent on the biochemical activities and rates of growth of the bacteria present in the milk.

The results of a preliminary series of observations showed that the "Period of sweetness" of a sample of milk at 15·5° C. agreed very closely with its reduction time (1 c.c. of a standard solution of methylene blue added to 40 c.c. of milk) at the same temperature, as is clearly indicated in Chart VI.

The use of the methylene-blue reductase test to replace the present keeping quality test would possess a very great advantage in that it would eliminate the personal factor of peculiarities in the sense of taste. Moreover, if desired, the observations could be made at very short intervals, and the inaccuracy of the keeping quality test as at present determined could be corrected.

The results of the preliminary observations were sufficiently good to warrant an extension of the study of the two tests. The work is still incomplete, but non-agreement has been very rare. Almost without exception it has been found that as soon as the reduction was complete a taint could be detected in the portion of milk reserved for tasting. At 15·5° C. changes take place at a rate which makes observations very convenient.



Summary.

Experiments with a modification of the keeping quality test for milk by the use of the methylene-blue reductase test at 15·5° C. are described. The results, so far as can be judged at present, indicate a close correlation between the appearance of taint as indicated by taste and the discharge of the colour of methylene blue.

This preliminary note is published in the hope that others who are interested will test the value of the suggestions for routine work.

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(*MS. received for publication August* 29, 1932.)

637.143:532.733

53. FACTORS AFFECTING THE SOLUBILITY OF MILK POWDERS

I. THE EFFECT OF HEAT ON THE SOLUBILITY OF MILK PROTEINS

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(With 8 Figures.)

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Introduction.

No reliable information is at present available with regard to the effect of process of manufacture on the solubility[1] of the protein of milk powders. Hunziker (1), it is true, gives a very well-balanced discussion of the subject,

[1] It should be made clear at the outset that the term "solubility," as applied to milk powders, is a misnomer. The lactose and part of the inorganic salts exhibit true molecular solubility. The milk proteins (and, in whole milk, the milk fat), on the other hand, are present in milk in colloidal suspension. Moreover, these are the constituents which give milk its unique physical properties. The coagulation of milk by rennet, by alcohol, and by heat are, for instance, primarily dependent on the properties of the milk proteins; the milk constituents which are in true solution play only a secondary part in such coagulation. The so-called "solubility" of milk powders is also primarily determined by the physical condition of the proteins, since such solubility is dependent on the ability of the proteins to undergo re-dispersion into a stable suspension. While, therefore, the term "solubility" is employed throughout this paper in describing the properties of milk powders, it should be understood that this term implies "suspension stability," and not true molecular solubility.

but it is apparent from his summary of the views of other workers that these are seldom based on adequate experimental evidence. In fact the only points on which there is general agreement among workers in this field are that the proteins of spray-dried powders are, on the whole, extremely soluble, while those of roller-dried powders are relatively insoluble. Moreover, even where experimental data are available, there remain many unexplained points. For example, Hunziker shows that the solubility of the proteins of a milk powder may vary according to the temperature at which the powder is reconstituted—a higher temperature giving a greater solubility. Such an observation cannot at present be brought into line with current theories regarding the properties of denatured proteins.

In view of the confused opinions which are at present held on the whole subject, and of the complete lack of information on the various factors involved, it appeared desirable to make a thorough investigation into the effect of the process of manufacture on the solubility of the dried product. Since, as stated above, roller-dried powders are markedly insoluble as compared with spray-dried powders, it was decided, as a first step, to consider in detail the nature of the heat treatment to which roller-dried powders are subjected during drying.

THE PROCESS OF ROLLER DRYING.

Fig. 1 represents the side elevation of a typical twin-cylinder roller drier. The technical construction and method of operation of this type of drier are dealt with in detail by Scott (2). It will be sufficient for the present purpose to outline the physical changes which may be expected to take place at the surface of the rolls and in the milk film itself.

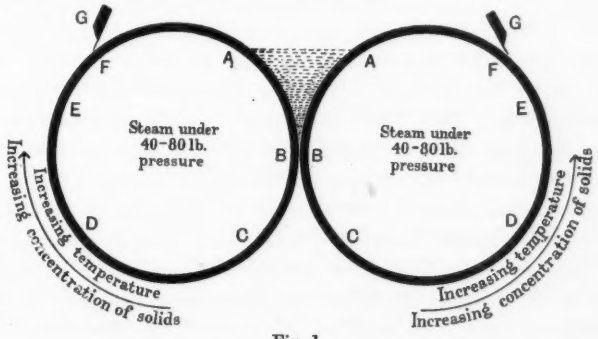

Fig. 1.

At *F* the dried film of milk is removed from each roll by means of a scraper *G*. Between *F* and *A*, therefore, the surface of the roll is free from any milk film, and will tend to acquire the full temperature of the hollow interior, which is heated with pressure steam to a temperature between 130 and 160° C. At *A* the metal surface of the roll comes into contact with the liquid milk lying in the trough, and between *A* and *B* heat will be lost from

this surface, with a consequent fall in the temperature of the metal. On the other hand the heat gained by the liquid milk will be sufficient to maintain active boiling in the trough.

The gap at *B* is adjusted to permit a thin film of the heated milk to be carried on the surface of the roll. Between *B* and *C* evaporation of water from this film proceeds, and we may assume (since the film is probably still liquid) that this evaporation takes place at the boiling-point of milk—a temperature only slightly above 100° C. Overheating between these two points is therefore unlikely to be encountered.

Between *C* and *D*, however, the evaporation will have resulted in the formation of a film having a relatively high percentage of solids, probably of a viscous nature. There will be a threefold effect: (1) the boiling-point of the film may be considerably higher than 100° C.; (2) the denaturing effect of high temperature in such a concentrated form of milk solids may be considerable; and (3) the viscous film may stick to the surface of the roll, with consequent over-heating. In addition, the temperature of the metal surface will tend to rise as it is further removed from the cooling effect of the liquid milk contained in the trough. The combined effects of these four factors may therefore result in definite over-heating of the film when the latter is in a concentrated, and possibly therefore an easily affected, state.

By the time that the film reaches *E* we may assume that it is virtually dry. The further heating which takes place between *E* and *F* may be termed "dry" heating, practically no further moisture being removed. It should be pointed out that this dry heating will take place at a relatively high temperature—approaching the full temperature of the roll.

In the above description the process of drying has been taken step by step, but it should be recognised that, in practice, the actual drying of the milk will be completed in a very short space of time—probably not more than 4 sec. Moreover, the various changes which occur have been indicated somewhat arbitrarily. We do not, in fact, at present possess information as to the actual changes in concentration of solids and in temperature which occur, under a given set of working conditions, at different parts of the roll. The description does, however, illustrate three important points: (1) During the first stage of evaporation, when the film is still in a liquid condition, and the concentration of milk solids is low, overheating, with consequent denaturation of the protein, is unlikely to take place. (2) The critical period of heat treatment may be expected to coincide with the formation of a viscous film containing a high percentage of solids. (3) Over-heating in a dry state must also be considered a factor of possible importance in determining the quality of the finished product.

In view of these conclusions, the work outlined in the present paper has been devoted to the elucidation of two main points; first, the effect of heat treatment on liquid milk and particularly on high concentrations of milk solids, and second, the effect of over-heating on the dried powder.

PART I. INSOLUBILITY INDUCED BY HEATING IN A LIQUID OR MOIST STATE.

In planning the first part of the work two main objectives have been kept in mind: first, to determine the influence of variations in the concentration of milk solids on the liability of the milk proteins to coagulation; and second, to ascertain the relationship between the temperature of heating and the time required to render a given percentage of the milk proteins insoluble. Much preliminary work was necessary before a satisfactory technique could be devised for this purpose. Ultimately the following methods were adopted:

Technique.

Preparation of milk concentrations.

Since it was necessary to employ concentrations of milk solids up to 85 per cent., and since it was essential to use a source of milk which would be identical for all concentrations, reconstituted spray-dried separated milk powder was used throughout the experiments. The sample of milk powder employed was prepared by the "Milkal" process (3) and had the following composition:

	%
Moisture	2·6
Fat	1·6
Casein	28·4
Albumin *plus* globulin	5·9
Non-protein nitrogen (× 6·38)...	2·6
Lactose, etc. (by difference)	50·9
Ash (containing CaO 1·9 %, P_2O_5 2·1 %, Cl 1·1 %) ...	8·0
	100·0

This sample of milk powder was over 99·5 per cent. soluble in cold water, and was easily reconstituted to any desired concentration.

Solubility determinations.

In order to secure reliable and reproducible results, the technique employed in solubility determinations was made as drastic as possible.

Each treated sample was diluted so that it contained the equivalent of 2 g. of milk solids in 20 c.c. of distilled water. The diluted mixture, contained in a Pyrex boiling tube (25 × 200 mm.) was stirred for 30 min. by means of a small double propeller revolving at 2000 R.P.M., the boiling tube being immersed in a small water-bath so that the temperature of reconstitution could be accurately regulated. (Unless otherwise stated, all samples were reconstituted at 20° C.) After stirring, the contents of the tube were immediately transferred to a 25 c.c. centrifuge tube, and centrifuged at 3000 R.P.M. for 15 min. The supernatant liquid was then removed by a siphon to a collecting tube, care being taken not to disturb the loose upper layer of the deposit.

Determinations of the constituents contained in the supernatant liquid were made by the following methods: *total protein*, by precipitation in hot

4 per cent. trichloracetic acid (the mixture being maintained at 60–65° C. for 30 min.), filtration, and determination of nitrogen in the precipitate; *casein*, by Moir's(4) method; *albumin plus globulin*, by trichloracetic acid precipitation of the casein filtrate; *non-protein nitrogen*, by determination of residual nitrogen in the filtrate from trichloracetic acid precipitation. All nitrogen determinations were carried out by the Kjeldahl method.

Since the amounts of these constituents in the supernatant liquid from the reconstituted sample of untreated milk powder were known, the percentage of each constituent rendered insoluble by any given treatment could be calculated simply as follows:

$$\text{Percentage of (say) protein rendered insoluble} = \frac{U - T}{U} \times 100,$$

where U represents the protein percentage in the supernatant liquid of the untreated sample, and T that in the treated sample.

Results.

(*a*) *Influence of concentration of milk solids.*

A number of small tubes were prepared, each containing 2 g. of milk solids made up with water to give concentrations of solids varying from 20 to 50 per cent. The tubes were securely corked, and immersed in a water bath at 100° C. for exactly 30 min. They were immediately cooled, reconstituted to standard dilution and centrifuged, and the percentage of protein rendered insoluble was determined as described above. The results are shown in the left-hand curve of Fig. 2.

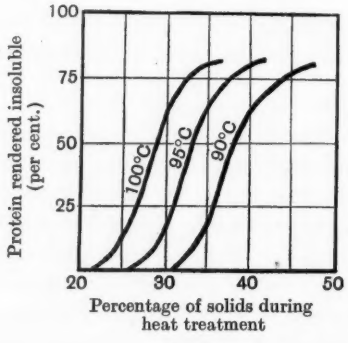

Fig. 2.

It will be seen that the percentage of protein rendered insoluble is markedly influenced by the percentage of milk solids present during heating. With a solution containing less than 20 per cent. solids, heating at 100° C. for 30 min. had no effect on solubility; on the other hand, with 35 per cent. solids present more than 75 per cent. of the protein is rendered insoluble. Between these two concentrations there is a relatively rapid increase in the proportion of

protein rendered insoluble. It should be mentioned here that, at intermediate points, the exact degree of insolubility of the protein is difficult to determine, owing to the fact that the centrifuged mixture tends to separate into three indefinite layers; a top layer of fairly clear liquid, an intermediate milky layer, and a poorly separated deposit. While the general form of the curves is easily gauged, the observer is apt to be confronted with occasional anomalous results. With a sufficient number of determinations, however, it is possible to determine with a considerable degree of accuracy the end-points and general shape of the curves.

Two further series of experiments were carried out in which the temperatures of heating were reduced to 95 and 90° C. respectively, the time of heating being kept constant at 30 min. Similar curves were obtained, except that these were, in each instance, shifted to the right by about 5 per cent. solids for each reduction of 5° C. in the temperature of heating. Thus at 100° C. for 30 min., 50 per cent. of the protein was rendered insoluble when a mixture containing 28 per cent. milk solids was heated; at 95° C. the same result was obtained when the mixture contained 33 per cent. solids; while at 90° C. the production of 50 per cent. insolubility required a concentration of 38 per cent. solids.

These results clearly demonstrate two facts: first, that, for a given temperature, the higher the concentration of milk solids the more easily is the protein rendered insoluble; and second, that, for a given time of heating, an increase of 1 per cent. in the concentration of solids lowers the temperature required to produce a given degree of insolubility by about 1° C.

The fact that insolubility is only induced in the lower concentrations after fairly prolonged heating at 100° C. indicates, however, that denaturation of the milk proteins will not take place in the preliminary stages of evaporation during manufacture—an observation which supports the statement made earlier in this paper that the critical period of heat treatment in roller-drying will probably coincide with much higher concentrations of solids. It was felt, therefore, that experiments should be carried out on samples having far higher solids content, and that an attempt should be made to determine the relation of time and temperature, as well as of milk solids concentration, to the production of insolubility.

Efforts were made to devise a method of working with samples which contained between 60 and 80 per cent. of milk solids, but these were found to be too tough and unpliable to handle, and the attempt was abandoned. A technique was, however, finally devised for obtaining a uniform sample of moistened milk powder which contained approximately 85 per cent. solids. While this concentration appeared at first sight to be extreme, the results of Lampitt and Bushill (5), in which the authors found a maximum rate of storage deterioration in samples of powder containing 12–14 per cent. moisture, provided some justification for using such a high concentration of solids.

(*b*) *Relation between temperature and time of heating.*

A bulk sample of moist milk powder was prepared by the following technique:

100 g. of the milk powder was spread in an even layer over a metal sheet of approximately 1500 sq. cm. Cold air which had previously been saturated with steam was passed over the powder for 1½ hours, the powder being stirred every 10 min. At the end of this period the whole of the powder was transferred to a large porcelain evaporating basin, and was thoroughly mixed by means of the rounded end of a glass flask. (It is important that the mixing surfaces should be smooth, otherwise "caking" of the moistened powder results.) The treated powder was at once transferred to a stoppered bottle, and kept in a refrigerator at 2–3° C. until used. In the experiments recorded in this paper, the powder was used within 3 hours of preparation.

Lampitt and Bushill(5) state that they experienced difficulty in securing homogeneous samples of even moisture content for their work. In order to ascertain how far the bulk of moistened powder used in the present experiments was homogeneous, moisture determinations were carried out in triplicate on 2 g. of the powder. The results gave values of 13·35, 13·34 and 13·32 per cent. of moisture.

In a preliminary series of experiments carried out with the moist powder, an attempt was made to determine the rate at which the protein was rendered insoluble at temperatures between 80 and 100° C. At these temperatures, however, the reaction was so rapid that accurate time measurements could not be made. Experiments were finally run at the following temperatures: 63, 51, 35, 22·5, and 15·7° C.

With the exception of the experiments at 63° C., which were carried out by the special technique detailed in the second part of this paper, all experiments were made as follows: A series of tubes was prepared, each containing 2·3 g. of the moist powder (equivalent to 2 g. of milk solids). The tubes were immersed in water baths at the above temperatures. At stated intervals, one or more tubes were removed and cooled, the contents were reconstituted to standard dilution and centrifuged, and the percentage of protein rendered insoluble was determined as before.

The detailed results are shown in the five graphs comprising Fig. 3. In these graphs the heavy black curves illustrate the progressive increase in the percentage of protein rendered insoluble as a result of increasing time of heat treatment. It will be seen that the curves ultimately reach a constant level, corresponding to the insolubility of about 75 per cent. of the total protein. Lampitt and Bushill(5) state that they have been unable to render the whole of the milk proteins insoluble by the storage of moist milk powder at room temperature or at 30° C., and that, while the soluble portion ultimately contains only a small proportion of the casein originally present, a relatively large proportion of the albumin and globulin remains in solution. In the present investigation the individual proteins were not determined, but

Protein rendered insoluble (per cent.)

the results show general agreement with those obtained by Lampitt and Bushill.

Moreover, when the time of heating is plotted against the logarithm of the residual (or soluble) protein, a straight line is obtained over that portion of the curve where the change in solubility is proceeding at a measurable rate. Similar curves have been obtained by Chick and Martin (6) in their studies of the denaturation of protein, so that it is reasonable to assume that, in the present instance, we are dealing with a true denaturation phenomenon. It is true that Chick and Martin were measuring the rate of reaction of a process which resulted in the flocculation of a protein from solution, whereas in the present paper the "solubility," or power of returning into colloid suspension, has been studied. These processes are, however, essentially of the same nature, since flocculation is actually due to the inability of the flocculated material either to remain in, *or to be re-dispersed into*, a stable suspension.

The most striking points illustrated in the curves are, however, the remarkably low temperatures at which this apparent denaturation takes place, and the relatively short periods of heating which are required. Thus, at

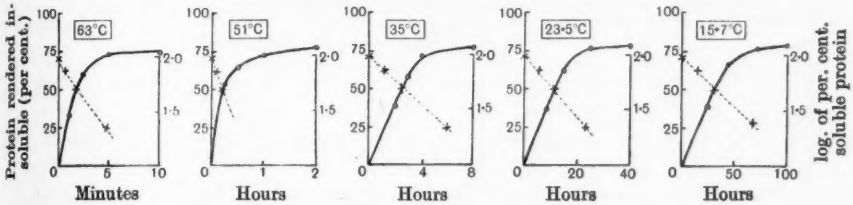

Fig. 3.

23·5° C., 50 per cent. of the protein is rendered insoluble in about 10 hours, at 35° C. $2\frac{1}{4}$ hours are required, at 51° C. about 15 min., while at 63° C. only 90 sec. are necessary.

In order to ascertain the exact relationship between time and temperature of heating, these points have been transferred to a single graph (Fig. 4), in which the temperature of heating (in ° C.) is plotted against the time of heating (expressed logarithmically in seconds) required to produce 50 per cent. protein insolubility. It will be seen that the points so plotted fall on a straight line (*AB*), the slope of which indicates that, for each rise in temperature of 10° C., the reaction velocity increases practically fivefold (actually 4·46).

Moreover, it will be seen that, by extending the line *AB* over the range of temperatures encountered in the roller-drying of milk (namely between 100 and 130° C.), the extrapolated time values are extremely low. Thus it appears that, at 100° C., the proteins of the moist powder would be rendered 50 per cent. insoluble in about half a second, while at 130° C. the same insolubility would be induced in less than one-hundredth of a second. A full discussion of the significance of these figures is included at the end of Part I of this paper.

(*c*) *General correlation of time-temperature-concentration curves.*

In the earlier results detailed in this paper, figures were presented which showed the relation of the concentration of milk solids to the temperature at which 50 per cent. protein insolubility was induced—the time of heating being kept constant at 30 min. It was decided to see how far these figures might be correlated with those available for the time-temperature relationships of the moist powder. For this purpose the three available points, corresponding to concentrations of 28, 33 and 38 per cent. milk solids, were plotted on Fig. 4. On the assumption that the time-temperature relationship is constant for all

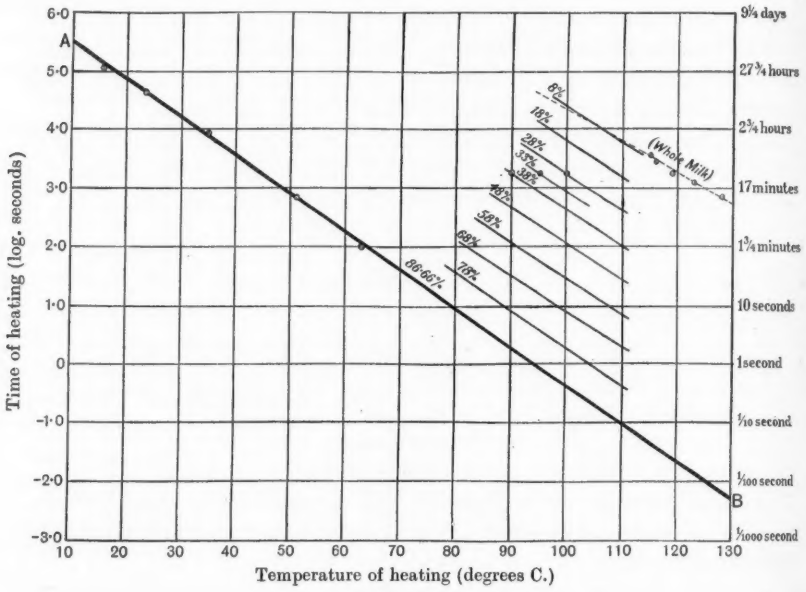

Fig. 4.

concentrations of solids, lines were drawn through each point, parallel to *AB*. The perpendicular distance between the three new lines was then measured, and additional lines were drawn corresponding to the theoretical values for 48, 58, 68, 78 and 88 per cent. milk solids. The fact that the latter line coincided almost exactly with the experimental line *AB* (86·66 per cent. solids) provides very strong evidence that the composite figure gives at least an approximate measure of the complete time-temperature-concentration relationships of the whole system.

Additional evidence is also provided in data taken from the paper by Holm, Deysher and Evans (7). These authors, who were investigating the coagulation of evaporated milks, found that a direct relationship existed between the temperature of coagulation and the time of heating, when the latter was expressed logarithmically. Typical results from their experiments

are plotted in Fig. 4, where they are shown by the dotted line. It will be seen that these results, which refer to good quality whole milk, coincide closely as regards both position and slope with those obtained by the present author. Considering that the experiments of Holm, Deysher and Evans were carried out on a different type of milk and by a technique differing materially from that described in this paper, the results show a remarkably close correlation.

Assuming then that Fig. 4 does provide an accurate measure of the time-temperature-concentration relationships of the whole system, it should be possible by extrapolation to obtain a reliable estimate of the effect of varying concentrations of milk solids on the production of insolubility at temperatures approximating those encountered in roller-drying. Table I shows, for instance, the relation between milk solids concentration and the production of insolubility at 100° C., which is the minimum temperature to which the milk is subjected during atmospheric roller drying. The figures show conclusively that no measurable insolubility will be induced by roller drying at this temperature until the milk solids have reached a concentration of 60 per cent.,

Table I. *Relation between milk solids concentration and time of heating at 100° C. required to induce 50 per cent. insolubility.*

Milk solids concentration %	Time of heating required
8	7½ hours
18	2 ,,
28	33 min.
38	8 ,,
48	2 ,,
58	31 sec.
68	8 ,,
78	2 ,,
88	0·4 ,,

but that there is a critical range of concentration between this and 88 per cent. solids at which insolubility will be induced extremely rapidly.

(*d*) *Reaction velocity at storage temperatures.*

The production of insolubility in milk powders which are stored in a moist condition has been demonstrated by numerous workers (8). The most common method employed has been to pass moist air over layers of the powder for stated periods on successive days, and to measure the increase in moisture content and the decrease in solubility which take place. In view of the fact that, in such experiments, one is dealing with a system whose water content is constantly increasing, it is impossible by such means to obtain any indication of the influence of concentration of solids on the reaction velocity at any given temperature.

Quite recently Lampitt and Bushill (5) have, however, published the results of an investigation in which the concentration of solids was kept approximately constant for each series of experiments. These authors showed that under such conditions there existed an optimum concentration of moisture

at which the reaction proceeded at a maximum rate, the concentration lying between 12 and 13 per cent.

The curve *AB* in Fig. 4 represents the time-temperature relationship of a milk powder containing 13·34 per cent. moisture, which approximates Lampitt and Bushill's optimum value. Two important conclusions may be drawn from the curve. In the first place it appears that the reaction which takes place at storage temperatures is identical in nature with that which takes place as a result of heat treatment during manufacture, since the experimental points for all temperatures fall on a straight line. Incidentally this also provides evidence that the insolubility which develops at storage temperatures is a purely physico-chemical reaction, and is not associated with bacterial multiplication in the moist powder. In the second place, the curve indicates the time-temperature relationship of the reaction at storage, as well as at higher, temperatures. It not only confirms the generally accepted statement that moist powders deteriorate more rapidly as the storage temperature is raised, but it provides an exact measure of the temperature coefficient of the reaction.

Discussion.

(*a*) *The nature of the insolubility induced by liquid or moist heating.*

In the previous pages evidence has been presented which indicates that the insolubility induced by liquid or moist heating is due to denaturation of the milk proteins, and particularly of the casein. The exact nature of the changes which occur is, however, not at present understood. The heat coagulation of a protein is usually ascribed to two independent reactions: first, denaturation itself, which is probably associated with some internal rearrangement in the protein molecule; and second, flocculation, which is due to interaction between the denatured protein and the ionic constituents of the system. There is every reason to believe that the production of an insoluble form of protein during the process of milk drying is identical in nature with the ordinary heat coagulation of a protein, such as occurs, for instance, in the manufacture of evaporated milk. Moreover, it appears likely that the combined influence of milk solids concentration and of heat treatment in rendering the protein insoluble is associated with the denaturation process itself, rather than with subsequent flocculation. The fact that, in the present work, differences were found in the solubility of powders which had been treated differently, but which had been reconstituted to standard solution (and therefore to a constant ionic concentration) supports this contention. On the other hand it is possible that the drying process may have affected certain ionised constituents (for instance, by precipitating calcium phosphate), so that this factor must also be taken into account. More information will obviously be required before a complete explanation of the reactions involved can be given.

Since the insolubility of the proteins of milk powder appears to be caused by factors identical with those responsible for the coagulation of evaporated

milk, an interesting possibility arises. It has been found possible to control the coagulation of evaporated milk by two means: first, by selecting milk on the basis of the alcohol test, which in general, eliminates those samples which are particularly liable to heat coagulation; and second, by the process of fore-warming the milk before manufacture, which has the effect of reducing the susceptibility of the milk to coagulation. Would the adoption of these methods be of value in controlling the solubility of milk powders? This question seems to indicate a promising field for useful inquiry.

(*b*) *Application of the results to commercial roller drying.*

It has already been mentioned that no reliable information is at present available with regard to the effect of process of manufacture on the solubility of the protein of milk powders. Moreover, it now appears that previous authors have entirely failed to take into consideration the two most important factors influencing protein insolubility, namely, the inter-relationship of temperature and time of heating, and the influence of concentration of milk solids. Thus Hunziker (1) is content to state, as regards the heat effect on casein, that "the casein of normal milk is not coagulated at temperatures below the boiling-point," but that "it coagulates when milk is heated under pressure to 130–140° C." It is true that Holm, Deysher and Evans (7), in an extremely valuable study of the factors affecting coagulation in evaporated milks, have demonstrated that a logarithmic relationship exists between temperature and time of heating, and that increasing concentrations of milk solids lower the temperature of coagulation. Their experiments were, however, limited to relatively low concentrations of milk solids (from 16 to 26 per cent.), and were not directly applicable to the conditions encountered in the manufacture of dried milk.

The experiments described in the present paper provide, in fact, the first definite evidence of the profound influence of these factors in controlling the solubility of the proteins of milk powders. They demonstrate conclusively that, at temperatures approximating 100° C., and at concentrations of milk solids between 60 and 90 per cent., insolubility may be induced by heating for periods varying between a few seconds and a fraction of a second. The extreme difficulty of producing an atmospheric roller-dried milk powder of a high degree of solubility is at once apparent, since, during drying, the heated milk solids are bound to pass through these ranges of temperature and concentration.

It may be argued that the above conclusions lack definite experimental evidence, since they are largely based on extrapolated data. The fact that the time-temperature relationships found in the present study are straight-line relationships, and that a very close correlation has been found between experiments carried out at widely different temperatures and with widely varying concentrations of milk solids, provides, however, very strong indirect evidence for the above conclusions. Moreover, additional experimental data has been

obtained which clearly demonstrates the ease with which milk proteins may be rendered insoluble at drying temperatures. For instance, a sample was prepared which contained 66 per cent. milk solids, and this was heated in a thin layer (as described in Part II of this paper) to a temperature of 103° C. for 10 sec. During this short period some evaporation took place, but the mass was still steaming when removed from the heater. Nevertheless, as a result of the heat treatment over 80 per cent. of the protein was rendered insoluble.

It appears, therefore, that the general conclusions outlined in this paper do have an important application in commercial roller drying. Far more extensive work will, however, be required before the conclusions can be applied to any given set of working conditions. This is due to two facts. In the first place the present work has been limited, for simplicity, to a study of separated milk. In practice it is more common to dry whole milk, and the presence of fat will introduce a complicating factor. Moreover, in the present study one bulk sample of separated milk powder has been used throughout, whereas it is possible that with other samples, containing, for instance, different proportions of mineral salts, the conditions governing the production of insolubility may be modified.

In the second place, our knowledge of conditions on the drying rolls is entirely inadequate. From the point of view of heat treatment, it is extremely important to know the temperature gradient on the rolls, and also the rate of evaporation at different points on the metal surfaces. At present we do not know how the rate of evaporation will vary with the concentration of the solids in the film, nor do we know, for instance, how the "gap" width will affect the thickness (and therefore the rate of evaporation) of the film. Again, we have no data on the boiling-point of high concentrations of milk solids, nor can we measure the cooling effect of evaporation on the milk film. Until these factors are elucidated, it will not be possible to assess the effect of any given set of manufacturing conditions on the solubility of the dried powder.

The present work does, however, provide a basis for a more rational study of the whole problem, and indicates the more important physico-chemical factors which must be taken into account in any detailed study of the subject.

Part II. Insolubility induced by heating in a dry state.

The statement has been frequently made that, while heating in a moist state will denature a protein, dry heating has no detrimental effect. Consequently it has been suggested (9) that, provided milk can be reduced to a dry state without affecting the solubility of the milk proteins, subsequent heating will do no harm. On the other hand it has been found (10) that the solubility of casein in acids is definitely modified by such dry heating. It therefore appeared desirable to investigate the effect of heating milk powders in a dry

state since, in both roller and spray drying, there is a risk that such heating will occur during commercial manufacture.

Preliminary experiments.

In preliminary experiments it was found that a definite alteration was, in fact, produced in the solubility of the casein as a result of heating milk powder in a dry state. Table II shows, for instance, the relative solubilities of the proteins of a sample of spray-dried powder after (*a*) no treatment, (*b*) heating in 10 per cent. solution for 40 hours at 100° C., and (*c*) heating in a dry state for 40 hours at 100° C. In each case the solubility was determined in both cold (20° C.) and hot (90° C.) water.

Before dealing with the general conclusions which may be drawn from this table, there is one special point to which attention should be drawn.

Table II. *The comparative effects of heating milk powder in a dry state and in solution.*

Heat treatment of milk powder	Reconstituted in water for 30 min. at	Constituents remaining in solution after centrifuging at 3000 R.P.M. for 15 min. Total solids %	Total protein %	Casein %	Albumin *plus* globulin %	Non-protein nitrogen %	Percentage of milk powder constituents rendered insoluble* Total solids	Total protein
None	20° C.	9·38	3·36	2·78	0·58	0·04	0	0
	90° C.	9·42	3·37	3·14	0·23	0·05	0	0
Reconstituted and heated in solution for 40 hours at 100° C.	20° C.†	6·10	0·21	0·11	0·10	0·13	35	94
	90° C.†	6·12	0·22	0·13	0·09	0·12	35	94
Heated in an open dish in a dry state for 40 hours at 100° C.	20° C.	7·17	1·43	0·92	0·51	0·05	24	57
	90° C.	9·32	3·28	2·91	0·37	0·05	1	2

* Assuming that untreated milk powder reconstituted at 20° C. is 100 per cent. soluble.

† As the powder had been reconstituted prior to heat treatment, further water was not added at this stage, but the solutions were stirred and heated exactly as in the other four experiments.

The spray-dried powder which was used in the experiments was completely soluble in cold water. Table II shows that the solubility of the total solids and of the total protein was identical whether the powder was reconstituted in cold or in hot water. On the other hand the casein shows an apparent increase in solubility in hot water equivalent to 0·36 per cent., while the albumin plus globulin fraction shows a decrease in solubility of 0·35 per cent. There is no doubt that these differences are apparent and not real. During reconstitution in hot water the solution was mechanically stirred at 2000 R.P.M. At 90° C. a large proportion of the albumin would normally have been coagulated, but it appears that the vigorous stirring caused some change in the condition of the protein which prevented coalescence of the protein particles, so that no deposit was formed on centrifuging. When, however, acetic acid was added to precipitate the casein, the latter carried down the albumin, and this resulted in an apparent increase in the casein content. Apart from the theoretical interest of this observation, it demonstrates that, in judging the differential solubility of a milk powder in cold and hot water, the criterion of

solubility must be based on the estimation of total protein, and not on that of casein. The latter would lead the observer to erroneous conclusions.

Turning now to the general conclusions which may be drawn from Table I, it will be seen that dry heating does produce a remarkable effect on the solubility of the milk powder. The solubility of the milk proteins, and particularly of the casein, is markedly reduced, the dry heating rendering 57 per cent. of the total protein, and 66 per cent. of the casein, insoluble in cold water. The solubility is, however, regained when the powder is reconstituted in hot water. This result is in marked contrast to that obtained by prolonged heating of the powder in solution, when the protein insolubility is irreversible. The nature of the insolubility induced by dry heating will be discussed later.

Since the preliminary experiments demonstrated that dry heating does affect milk powders, it was decided to make a detailed study of the time-temperature relationships of the reaction, particularly at temperatures approximating those encountered in commercial milk drying. For this purpose the following technique was devised.

Technique.

It was essential that the technique should allow for very accurate measurements of the temperatures and times of heating, and, in particular, that the

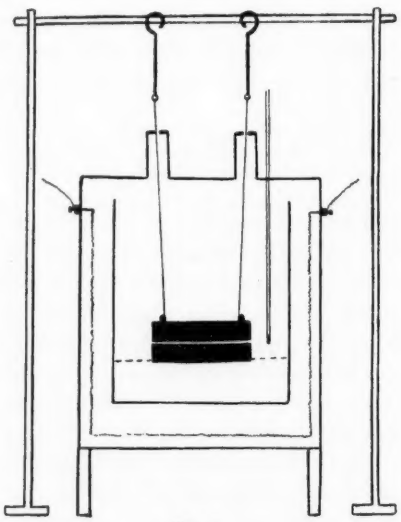

Fig. 5.

method of applying heat should ensure an immediate rise in the temperature of the powder to the desired level. With this object, a special heating apparatus, illustrated in Fig. 5, was devised.

Two blocks of wrought iron, each weighing about 3 kg., were contained

in an electrically heated air oven, capable of adjustment to any temperature between 100 and 150° C. One block was placed on the oven shelf, and the other was suspended above it by a wire attachment, the wires being carried through the top of the oven and fixed to hooks. By this means the position of the upper block could be regulated by the observer without opening the oven door.

The two adjacent faces of the blocks were carefully machined to give an even heating surface. When not in use the two blocks were kept with their faces about 1 cm. apart, and before any series of experiments was carried out, the whole system was retained at the desired temperature for at least 24 hours. By this means sufficient time was allowed to ensure the thorough heating of the blocks.

In carrying out each experiment, 2·05 g. of spray-dried powder (identical with that used in the experiments described in Part I), containing 2 g. of milk solids, were spread in an even layer, covering about 30 sq. cm., between two thin sheets of copper foil. The door of the oven was opened, the copper sheets were quickly inserted between the heated iron blocks, the upper block was immediately lowered on to the sheets, and the oven door was closed. Since the conductivity of copper is high, the heat from the blocks was very rapidly transferred to the layer of powder between the sheets. After the requisite heat treatment, the sheets were removed from the oven, the powder was transferred to a boiling tube, reconstituted to standard dilution in cold water and centrifuged, and the solubility determined as outlined in Part I of this paper.

Experiments were carried out at the following temperatures: 100, 117, 123, 129 and 139° C.

Results.

(*a*) *Solubility of casein.*

The progressive increase in insolubility of the casein which results from dry heating is illustrated in the five graphs comprising Fig. 6. It will be seen

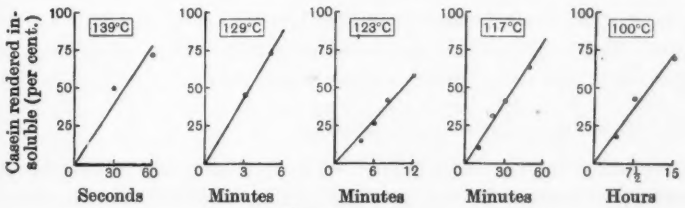

Fig. 6.

that, for each temperature studied, the points fall on a straight line, indicating that the rate of production of insolubility is directly proportional to the time of heating. Moreover, these curves also show the very great increase in the

velocity of the reaction with increasing temperature. Thus, at 100° C. the casein is rendered 50 per cent. insoluble in 10 hours, at 117° C. 35 min. are required, at 123° C. about 10 min., at 129° C. about 3 min., while at 139° C. only 40 sec. are necessary. When these values are transferred to a graph (Fig. 7) in which temperature of heating (in ° C.) is plotted against time of heating (expressed logarithmically in seconds) it will be seen that the points fall on a straight line, the slope of which indicates that, for each rise in temperature of 10° C., the reaction velocity increases over sixfold (actually 6·32).

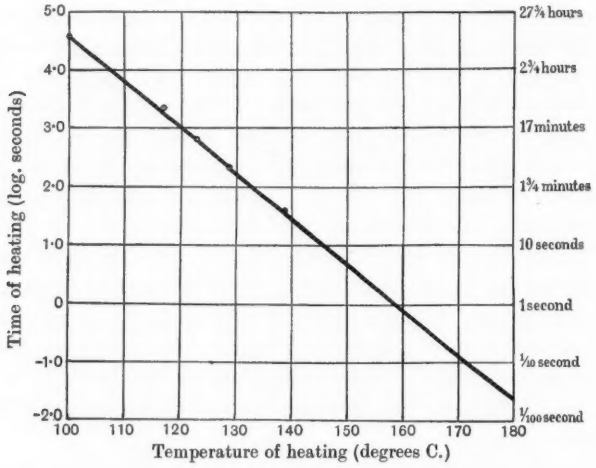

Fig. 7.

(*b*) *Solubility of other nitrogenous constituents.*

Neither the albumin plus globulin fraction, nor the non-protein nitrogen fraction showed any marked alterations as a result of the dry heat treatment. For instance, in four observations made at 117° C., the concentrations of the former fraction remained constant at 0·52, 0·52, 0·51 and 0·52 per cent., while the non-protein nitrogen values were 0·081, 0·078, 0·082 and 0·074 per cent. The casein appears, therefore, to be the only nitrogenous constituent of the milk powder whose solubility is progressively decreased.

(*c*) *Solubility of non-nitrogenous constituents.*

A number of determinations of the solubility of the total solids were made, in order to ascertain whether any of the milk constituents other than the casein were rendered insoluble. The results are shown in Fig. 8, where the percentages of non-nitrogenous constituents rendered insoluble are plotted against the percentages of total solids rendered insoluble. It will be seen that there is definite evidence of a progressive decrease in the solubility of the non-nitrogenous constituents as the solubility of the total solids decreases—

that is, as the heat treatment of the milk powder becomes more drastic. The decrease in solubility is small, amounting even with the most drastic heating to only 7 per cent. of the non-nitrogenous constituents. It is possible that it is due to an alteration in the lactose, since the treated milk powders show a progressive "browning" or caramelisation. More work is, however, required to settle this point.

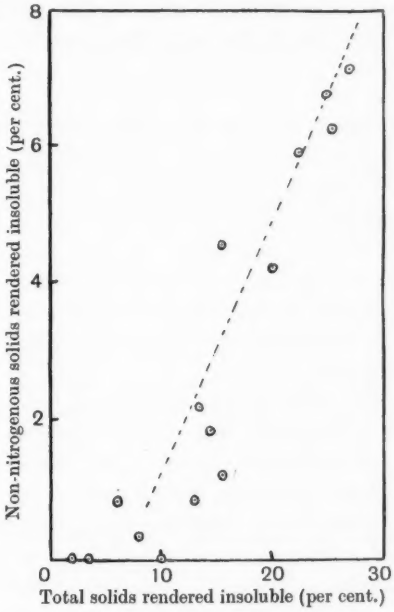

Fig. 8.

Discussion.

(*a*) *The nature of the insolubility induced by dry heating.*

It has already been made clear that the increased insolubility which results from dry heating is almost entirely associated with a change in the properties of the casein. The form of the curves shown in Fig. 6 indicates that the change is physical, rather than chemical, in character, the change in solubility being directly proportional to the time of heating, but independent of the quantity of unchanged casein. Such a result is analogous to that which would be obtained, for instance, in the evaporation of water, where the application of equal amounts of latent heat will produce equal amounts of evaporation, independent of the quantity of water remaining unchanged. It appears possible, indeed, that the insolubility is in fact caused by some type of dehydration effect—as, for instance, the removal of water of imbibition from the protein particles. Such a hypothesis would provide an explanation for the observed

effect of hot water in restoring the solubility of the protein, as shown in Table II.

Additional evidence in support of this hypothesis has been obtained from a study of the dehydrating effect of absolute alcohol on the dry milk powder. Duplicate samples of 2 g. of powder were treated with 40 c.c. of cold absolute alcohol for 48 hours, and the solubility of the treated samples was determined in both cold and hot water. In cold water only 45 per cent. of the casein was found to be soluble, but in hot water the whole of the casein returned into solution.

(*b*) *Application of the results to commercial drying processes.*

It has already been mentioned that the solubility of roller-dried milk powders may vary according to the temperature at which they are reconstituted. Thus in one roller-dried sample examined by the author, only 34 per cent. of the protein was found to be soluble when the powder was reconstituted in water at 20° C., while 52 per cent. was soluble in water held at 90° C. From the results obtained above, it appears that this differential solubility may be associated with the period of dry heating which is liable to take place in the later stages of roller drying.

Two arguments may, however, be advanced against this explanation. In the first place, it is undoubtedly true that the phenomenon of differential solubility is found in powders which contain appreciable quantities of moisture: the sample quoted above contained, for instance, 2·7 per cent. of moisture. How, then, can the differential solubility be considered a dehydration process? The only explanation which can be advanced is that we are dealing, in milk powders, with a complex system, and that dehydration of one constituent (*e.g.* casein) may be accompanied by readjustments in the internal phases of the system. In the second place, it appears that powders which exhibit differential solubility do not, during manufacture, undergo dry heating for the relatively long periods indicated in Fig. 7. Thus at 130° C., which is the maximum temperature employed in some roller-drying plants, it would, according to Fig. 7, take $1\frac{3}{4}$ min. to induce 50 per cent. insolubility of the protein, whereas we know that the powder cannot in fact be subjected to dry heating on the rolls for more than a fraction of a second. On the other hand it should be pointed out that the experiments on which Fig. 7 is based were carried out on relatively thick layers of powder, and that dehydration may conceivably take place far more rapidly when the powder is in the form of a thin film such as would be obtained with a commercial drying plant.

It appears therefore that over-heating of the milk powder in a dry state may account for the differential solubility observed in roller-dried milk powders. With regard to spray-dried powders, over-heating in a dry state is also possible, especially where the powder is allowed to remain in the drying chamber for prolonged periods, or where, to increase thermal efficiency, high air inlet temperatures are used. Scott (11) has shown, on theoretical grounds,

that during the actual drying of the milk the cooling effect of evaporation will prevent over-heating of the particles. Once the powder is dry, however, this cooling effect is no longer available, and the powder will quickly be raised to the temperature of the surrounding air. While the risk of over-heating the dried powder is, in spray-drying, not considerable, it is nevertheless a factor which must definitely be taken into account in determining the thermal conditions and the design of spray-drying plants.

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(*MS. received for publication, July* 23, 1932.)

637.127:546.17

54. THE DISTRIBUTION OF NITROGEN IN MILK WITH REFERENCE TO THE SOLIDS-NOT-FAT CONTENT

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RICHMOND (1) states that the average protein distribution in cow's milk is 3·00 per cent. casein, and 0·40 per cent. albumin plus globulin, whilst van Slyke (2) gives 2·5 per cent. casein and 0·7 per cent. albumin plus globulin. Tocher (3) gives the range 1·654–4·083 (average 2·424) per cent. casein and 0·447–1·404 (average 0·740) per cent. albumin plus globulin. Albumin plus globulin has been termed the "albumin fraction," and beyond the statement that "the fraction is undoubtedly all albumin except for a fraction of a per cent. of globulin" (Albery (4)), no comprehensive data of the distribution of these proteins have been supplied. A difficulty in interpreting such data as exist arises from the fact that they have been secured by different analytical methods, but Moir (5) has applied reliable methods of protein separation to milk proteins, thus affording a standard method of analysis; but he used only one type of milk, and no complete nitrogen distributions in a range of samples are described in the literature.

During the winters of 1930 and 1931, studies in the composition of milk samples persistently low in solids-not-fat from certain herds in the Reading province were carried out, attention being paid to the constituents responsible for the poor quality. During the first winter it was found that a low lactose content (in samples from the British Friesian herd studied) was mostly responsible, but during the second winter the scope of the investigations was widened and the nitrogen distribution in a series of morning samples ranging in solids-not-fat content from 6·5 to 9·0 per cent. from a variety of sources was determined. Determinations of the chloride content were also made.

ANALYTICAL METHODS.

The separation of the milk proteins was carried out by the methods advocated by Moir (5), except that the precipitates were washed with solutions of the precipitating agents in the same concentrations as used for precipitating. This was found to expedite filtration. The following nitrogen fractions were determined: total, protein, casein, casein plus globulin; from these the amount of nitrogen in each individual group (protein, non-protein, casein, albumin and globulin) were calculated. Chlorides were determined by an open Carius method (Davies (6)). The results were the mean of duplicates, which always agreed closely.

DESCRIPTION OF SAMPLES.

The following range of samples was analysed:

(*a*) *Type milk.* These consisted of bulk samples from the typical herd of ten Shorthorn cows used for experiments at this Institute (7).

(*b*) *Bulk milk.* A series of advisory samples from Kent.

(*c*) *British Friesian.* Series of samples from six cows (A–F) which at the commencement gave milk containing the following percentages of solids-not-fat: 6·59, 6·96, 7·77, 7·82, 8·64, 8·94 respectively.

(*d*) *Ayrshire.* Series of samples from three cows (X–Z), which at the commencement contained 8·03, 8·61, 9·52 per cent. of solids-not-fat respectively.

(*e*) *British Friesian.* A series of samples from one cow (M) removed from its home farm to different conditions of management into the herd of the University of Reading. The average content of solids-not-fat was 8·1 per cent.

RESULTS.

Table I gives the results obtained for the nitrogen and protein distribution in the series of "type milk" samples (*a*), and the "bulk milk" samples (*b*). The results for the ranges of samples from individual cows (groups (*c*), (*d*) and (*e*)) are given in Table II. The mean of each range is given together with the standard deviation from the mean.

DISCUSSION OF RESULTS.

It will be convenient to adopt as a *normal* type of nitrogen distribution that for good quality milk showing uniform values over a considerable period. For this purpose the results of the "type milk" samples (Table I) are selected, since the nitrogen distributions in seven samples spread over a period of months show uniform values. The regular and desirable type of nitrogen distribution shown in these samples thus forms a basis for discussing the results for the individual samples.

Total nitrogen.

The *normal* value was approximately 0·5 per cent. Samples low in solids-not-fat were also low in total nitrogen, and those of high solids-not-fat were high. Generally a high percentage of chlorine was associated with a low nitrogen content. A variation of from 0·4 to 0·6 per cent. nitrogen was met with. Samples high in solids-not-fat were more constant in nitrogen content, the values for those low in solids-not-fat fluctuating between consecutive samples.

Non-protein nitrogen.

The conception of the constancy of the osmotic pressure of milk demands that for high solids-not-fat content the number of the simpler water-soluble molecules be low. Therefore, high solids-not-fat content must necessarily be

Table I. *Nitrogen and protein distributions in samples of morning milk.*

	"Type milk" samples (*a*)								"Bulk milk" samples (*b*)				
	8. xii. 31	26. i. 32	8. iii. 32	5. iv. 32	10. v. 32	24. v. 32	7. vi. 32	Mean	29. x. 31	9. iii. 32	10. iii. 32	15. x. 31	4. v. 32
% of milk:													
Fat	3·60	3·45	3·00	3·30	3·10	3·30	4·20	3·42 ±0·37	2·45	2·75	2·80	2·60	—
Solids-not-fat	9·02	9·10	8·98	9·05	8·92	8·98	8·65	8·96 ±0·17	8·88	8·54	8·83	9·10	—
Total nitrogen	0·516	0·497	0·492	0·466	0·506	0·506	0·495	0·497 ±0·015	0·508	0·448	0·464	0·519	0·472
Protein	3·07	3·04	2·97	2·78	3·02	2·91	2·97	2·97 ±0·08	2·98	2·54	2·60	3·00	2·73
Casein	2·49	2·44	2·40	2·24	2·49	2·42	2·39	2·41 ±0·08	2·53	2·14	2·19	2·64	2·19
Albumin and globulin	0·58	0·60	0·57	0·54	0·53	0·49	0·59	0·56 ±0·04	0·45	0·40	0·41	0·36	0·54
Albumin	0·39	0·39	0·39	0·37	0·37	0·35	0·43	0·38 ±0·02	—	—	—	—	0·27
Globulin	0·19	0·21	0·18	0·17	0·16	0·14	0·16	0·17 ±0·02	—	—	—	—	0·27
Chlorine	0·084	0·072	0·072	0·074	0·077	0·077	0·080	0·077 ±0·004	0·074	0·106	0·106	0·078	0·073
% of total nitrogen:													
Protein	93·4	95·9	94·5	93·7	93·5	93·5	94·1	94·1 ±0·8	92·0	88·7	87·8	90·5	90·8
Casein N	75·7	77·0	76·3	75·4	77·1	75·9	75·6	76·1 ±0·6	77·9	74·8	74·0	79·6	72·9
Albumin and globulin N	17·9	19·0	18·2	18·3	16·5	17·6	18·5	18·0 ±0·7	14·1	13·9	13·8	10·9	17·9
Albumin N	11·9	12·4	12·4	12·4	11·6	13·9	13·6	12·6 ±0·8	—	—	—	—	8·9
Globulin N	6·0	6·6	5·8	5·9	4·9	3·7	4·9	5·4 ±0·9	—	—	—	—	9·0
Non-protein N	6·6	4·1	5·5	6·3	6·5	6·5	5·9	5·9 ±0·8	8·0	11·3	12·2	9·5	9·2

Table II. *Nitrogen and protein distributions in morning samples from individual cows. A to F, M, British Friesian; X–Z, Ayrshires. Mean of series of samples with standard deviation.*

Cow	A	B	C	D	E	F	M	X	Y	Z
No. of samples ...	6	4	5	3	5	4	8	5	5	5
% of milk:										
Fat	1·84 ±0·34	2·51 ±0·40	2·46 ±0·33	3·22 ±0·06	2·67 ±0·24	2·79 ±0·10	2·53 ±0·10	2·63 ±0·31	3·42 ±0·57	4·17 ±0·19
Solids-not-fat	6·43 ±0·26	6·79 ±0·16	7·48 ±0·33	7·85 ±0·20	8·90 ±0·18	8·99 ±0·10	8·08 ±0·04	8·00 ±0·15	8·98 ±0·22	9·59 ±0·17
Chlorine	0·203 ±0·014	0·190 ±0·008	0·151 ±0·021	0·157 ±0·002	0·092 ±0·005	0·072 ±0·003	0·124 ±0·005	0·108 ±0·009	0·094 ±0·009	0·072 ±0·006
Total nitrogen	0·450 ±0·026	0·450 ±0·022	0·450 ±0·015	0·508 ±0·012	0·530 ±0·015	0·501 ±0·009	0·469 ±0·009	0·436 ±0·005	0·552 ±0·021	0·609 ±0·017
Total protein	2·36 ±0·26	2·56 ±0·15	2·50 ±0·05	2·94 ±0·08	3·11 ±0·12	3·05 ±0·06	2·75 ±0·06	2·53 ±0·05	3·28 ±0·15	3·68 ±0·12
Casein	1·42 ±0·19	1·69 ±0·14	1·76 ±0·09	2·10 ±0·02	2·37 ±0·12	2·45 ±0·13	2·20 ±0·09	1·90 ±0·07	2·57 ±0·11	3·06 ±0·09
Albumin and globulin	0·94 ±0·13	0·87 ±0·17	0·74 ±0·05	0·84 ±0·06	0·74 ±0·09	0·59 ±0·08	0·55 ±0·06	0·63 ±0·03	0·66 ±0·11	0·64 ±0·03
Albumin	0·44 ±0·07	0·57 ±0·11	0·37 ±0·09	0·48 ±0·05	0·49 ±0·08	0·45 ±0·08	0·35 ±0·05	0·37 ±0·08	0·44 ±0·10	0·39 ±0·03
Globulin	0·42 ±0·12	0·25 ±0·06	0·37 ±0·12	0·36 ±0·08	0·25 ±0·07	0·14 ±0·04	0·18 ±0·02	0·26 ±0·09	0·26 ±0·07	0·24 ±0·06
% of total nitrogen:										
Protein N	85·2 ±6·6	89·2 ±3·9	87·1 ±2·0	90·7 ±1·4	92·1 ±1·8	94·8 ±1·2	92·0 ±1·2	91·0 ±1·7	93·3 ±1·1	94·7 ±0·5
Casein N	51·4 ±6·6	58·2 ±3·1	61·4 ±1·4	64·8 ±1·0	70·1 ±2·3	76·6 ±3·8	73·9 ±2·0	68·5 ±2·7	75·3 ±2·9	78·4 ±0·2
Albumin and globulin N	33·8 ±4·0	31·0 ±5·2	25·8 ±2·4	26·0 ±1·3	21·9 ±2·7	18·3 ±2·6	18·5 ±2·3	22·6 ±0·8	20·0 ±1·4	16·2 ±0·8
Albumin N	16·4 ±3·0	17·7 ±2·6	12·9 ±3·1	14·9 ±1·4	14·2 ±2·3	14·1 ±2·4	11·8 ±1·5	13·4 ±2·9	12·2 ±2·6	10·2 ±1·1
Globulin N	15·7 ±3·6	14·4 ±0·6	12·9 ±4·4	11·1 ±2·4	7·5 ±2·0	4·2 ±1·4	5·9 ±0·8	9·2 ±3·3	7·8 ±1·8	6·2 ±1·4
Non-protein N	14·8 ±6·6	10·8 ±3·9	12·9 ±2·0	9·3 ±1·4	7·9 ±1·8	5·2 ±1·2	8·0 ±1·2	9·0 ±1·7	6·7 ±1·1	5·3 ±0·5

accompanied by the greatest amount of nitrogen in the form of *protein* nitrogen, *i.e.* minimum non-protein nitrogen values would be expected. The results in Tables I and II bear this out.

Samples high in solids-not-fat showed only 6 per cent. or less of the total nitrogen as non-protein nitrogen, which is the *normal* value. As the solids-not-fat decreased the non-protein nitrogen increased (up to 15 per cent. of the total nitrogen). The extra amount of non-protein nitrogen did not make up for the lowness in casein nitrogen. The connection between non-protein and globulin nitrogen which has generally been assumed was only observed for milk samples containing over 8·0 per cent. solids-not-fat.

Protein nitrogen.

In the normal and high solids-not-fat samples this accounted for 94 per cent. of the total nitrogen, but with lowering of solids-not-fat this figure fell to a minimum of 85 per cent. The protein content of milk low in solids-not-fat was therefore low for two reasons: (*a*) lowness in total nitrogen, and (*b*) a lower percentage of the nitrogen as protein. Fluctuation in protein nitrogen was greater in the low solids-not-fat samples.

Casein nitrogen.

The *normal* samples contained 2·4 per cent. casein. The standard given by Richmond (1) (3 per cent.) was reached only in one series. The fat/casein ratio was 1·4:1 for the *normal* samples, the ratio increasing to 1·5–1·6:1 for low non-fatty solid samples.

Casein nitrogen uniformly accounted for 76 per cent. of the total nitrogen in the *normal* samples and for 80 per cent. of the protein nitrogen. These figures were equalled and exceeded by the individual samples high in solids-not-fat. As the solids-not-fat decreased, the percentage of total nitrogen as casein nitrogen decreased and showed lack of constancy between consecutive samples. It was evident from the complementary nature of the figures for casein and non-protein nitrogen that the quality of the milk was better when the casein nitrogen was high and the non-protein nitrogen was low.

Albumin and globulin nitrogen.

Albumin accounted for 12·6 per cent. and globulin for 6·3 per cent. of the total nitrogen, and 13·4 and 6·7 per cent. respectively of the protein nitrogen in the *normal* samples. Samples from individual cows even when high in solids-not-fat diverged greatly from this ratio. This was particularly true of globulin nitrogen which exceeded 6·3 per cent. in many cases, and varied with consecutive samples. The maximum variability appears to occur in the samples lowest in solids-not-fat.

The albumin content of individual samples varied from 0·26 to 0·68 per cent., and the globulin from 0·08 to 0·57 per cent.

The protein distribution.

The published protein distribution in milk (casein 3 per cent.; albumin 0·5 per cent., globulin 0·2 per cent.) demands a casein/albumin/globulin ratio of 15:2·5:1. In the present study the *normal* samples uniformly showed a ratio of 14·2:2·3:1. Samples high in solids-not-fat approached this ratio but in poorer samples the ratios of both casein and albumin to globulin decreased. For 8·0 per cent. solids-not-fat, the ratio was 8·0:1·4:1 (15 samples) and for 6·5 per cent. solids-not-fat 3·4:1·0:1 (five samples).

Samples low in solids-not-fat decreased in casein content by roughly 25 per cent. of the *normal* casein content for every 1 per cent. fall in solids-not-fat below 9 per cent. The albumin tended to decrease with the casein, but naturally the rate of decrease was less.

SUMMARY.

The nitrogen distributions in 62 samples of whole morning milk covering a range of solids-not-fat content from 6·5 to 9·0 per cent. are discussed.

Samples high in solids-not-fat showed greater constancy in nitrogen distribution over a consecutive range of samples than those low in solids-not-fat.

Rich samples were characterised by high total nitrogen, low non-protein nitrogen and high casein nitrogen. Poor samples were characterised by low total nitrogen, high and variable non-protein nitrogen, low casein and consequently high albumin and globulin nitrogen. In samples low in solids-not-fat the albumin and globulin distribution was variable.

The casein/albumin/globulin ratio was roughly 14:2·5:1 for samples high in solids-not-fat, but the casein dropped in its proportion to globulin as the solids-not-fat decreased and the albumin showed also a slight parallel decrease.

The samples low in solids-not-fat which were studied appeared to be abnormal secretions, since they showed increased albumin, globulin and chloride contents.

The thanks of the author are due to C. E. Lesser and A. Wagstaff for the figures for fat and solids-not-fat.

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(*MS. received for publication September* 6, 1932.)

637.322:546.33.13

55. THE DISTRIBUTION OF SALT IN CHEDDAR CHEESE

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(With 3 Graphs.)

I. Variation in salt content in different cheeses from one vat.

During the course of an extended experiment on the rate of ripening of cheese, analyses were made of the salt and moisture content of each individual cheese from 40 vats of milk, comprising a total of 360 cheeses. The analyses were made 14 days after the date of manufacture. Moisture estimations were carried out by the oven method (1), and salt estimations by the method of McDowall and Whelan (2) on a sample plug of each cheese.

It was found that satisfactory duplicates for salt content could not be obtained when the cheese sample was prepared according to the method commonly used in factories and grading stores, viz. division by means of a knife into small cubes. When the cheese plug was ground to a paste in a mortar, good duplicates were regularly obtainable.

The results are summarised in Table I.

Table I. *Showing maximum, minimum and average range of variations in the figures for salt content and moisture content of nine cheeses from one vat. Results from analyses of cheese from 40 vats of milk.*

	Moisture	Salt
Maximum range of variation in nine cheeses from one vat	1·8	0·57
Minimum range of variation in nine cheeses from one vat	0·2	0·09
Average range of variation in nine cheeses from one vat	1·2	0·36

The "range of variation" represents the difference between the maximum and minimum figures for percentage of moisture or salt for the cheeses from one vat. It will be seen that there was recorded in one case a difference of 1·8 between the maximum and minimum moisture content of nine cheeses made under apparently identical conditions, the average difference being 1·2 per cent. of the weight of the cheese.

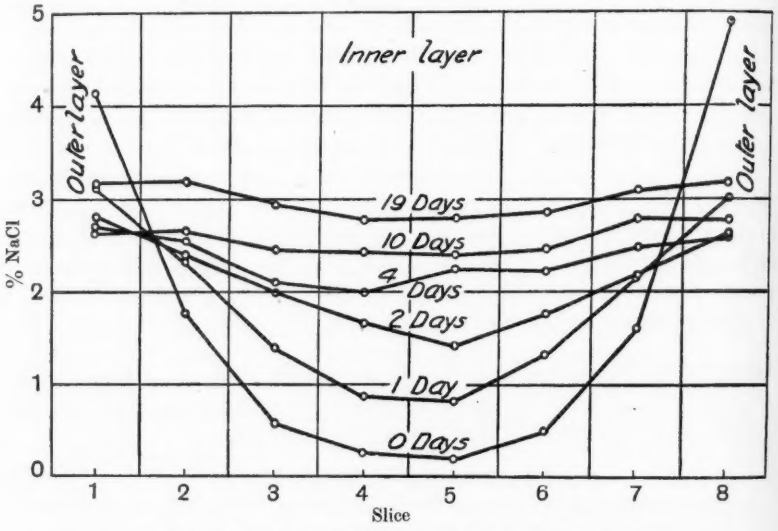
In cheeses from another vat the salt content varied from 0·97 to 1·54 per cent. of the weight of the cheese, representing a range of variation of 0·57 in the figure for the percentage of salt. There was no very definite correlation between variability of moisture content and variability of salt content. Further work has shown that variations of the same order may be encountered in samples taken from different positions in a single cheese. The fact

that good duplicate results for salt content were not obtainable when a single plug of cheese was cut up into small cubes for sampling is sufficient evidence to show that salt is not evenly distributed throughout a single cheese; and the above results show that the average salt content of a plug of cheese from one vat is not a very accurate measure of the average salt content of similar samples taken from other cheeses in the same vat.

Any figures for salt content of cheese, therefore, must be interpreted in the light of these facts, consideration being given to the method adopted for sampling and for preparation of the samples for analysis.

II. Diffusion of salt in Cheddar cheese.

When cheeses are salted by the indirect method in a brine bath, the distribution of salt throughout the body of the cheese takes place by the process



Graph 1. Salt diffusion in Limburger cheese in relation to time.

of diffusion. Orla-Jensen (3) has shown that the outer portions of Emmenthal cheese salted in this way are richer in salt than the inner portions; and he attributes to this fact the greater prevalence of gas-holes in the inner portions of the cheese.

Mrozek (4) has investigated the diffusion of salt into the body of Limburger cheese. Limburger cheeses, half-fat and full-fat cheeses, respectively, immediately after manufacture were placed in a 15 per cent. solution of sodium chloride for 24 hours. The cheeses were then removed from the brine and analysed at intervals for salt. An external layer, 2 cm. deep was removed from the cheese and discarded. The remainder was cut into eight discs, discs 1 and 8 being the external faces, and discs 4 and 5 the central portions. Mrozek's results for full fat cheese are shown in Fig. 1. The results for the

half-fat cheese were not markedly different. Fig. 1 shows that there is a rapid movement of salt within the cheese, and within 4 days the salt content is fairly uniform throughout the cheese. Mrozek noted at the same time a very considerable outward flow of water. During the first 4 days the average moisture content of the cheese fell from approximately 124 parts to approximately 103 parts per 100 parts of salt-free dried cheese.

These results for Limburger cheese appeared to be very much at variance with the results described under section I of this paper. With diffusion of equal rapidity in Cheddar cheese, it would be expected that the salt content would not show irregularities such as those quoted. The higher moisture content of Limburger cheese, it was realised, undoubtedly would permit a freer diffusion of salt, as also a more rapid movement of moisture from the centre to the outside, than would be the case with Cheddar cheese.

No measurements of the rate of diffusion of salt in Cheddar cheese have been recorded.

Experimental.

A 10 lb. unsalted Cheddar cheese was completely immersed the day after manufacture (*i.e.* immediately on removal from the hoop) in a 5 per cent. sodium chloride solution at room temperature for 130 hours. The cheese was removed from the solution and allowed to drain for 10 min.: it was then sampled for salt and moisture estimations. For each sample two plugs were taken close together, the length of the plug reaching just to the centre of the cheese. Samples were taken at the top centre, at the bottom centre, and at opposite points at middle height on the sides. Each of the two neighbouring plugs (4 in. in length) was divided into four equal parts, and the sections were numbered 1 to 4, the outer part containing the rind being No. 1, and the centre part No. 4. Corresponding sections from the two plugs were ground up together in a mortar, and cut into small pieces with a palette knife. Salt and moisture estimations were carried out on the prepared samples. The results are given in Table II.

It is evident that there has been a diffusion of salt, but the rate is slow compared with that cited by Mrozek for Limburger cheese.

It will be noted that the moisture content of the cheese was high, due to the fact that no salt was added during manufacture. Thus it could be expected that the rate of diffusion indicated was higher than is usual for a normal Cheddar cheese. Apart from this an abnormality was introduced due to the softening of the outside of the cheese by the immersion in brine. A further experiment, therefore, was carried out as follows:

Sufficient uncoloured curd was set aside, at the salting stage, from 10 lb. loaf cheeses. This quantity of curd was divided into two equal portions. To one portion salt was added in the ordinary way: the other portion was coloured by washing in a dilute solution of annatto, and was not salted. The two coloured, and the two uncoloured cheeses, were pressed for 3 hours in the

ordinary way. They were then cut into halves in a plane parallel to the ends, and an uncoloured and a coloured half were pressed together for 2 days. The resulting cheeses were kept in a curing room at 60° F. during the whole ripening period, and were turned daily. When the cheeses were cut it was possible to divide the original unsalted and salted portions exactly, using the annatto colour as an indication of the dividing line. It should be mentioned here that the technique had already been adopted at the Institute in other experimental work, and had been found to have no influence on the quality of the cheese.

At five stages during the period of ripening analyses were made of a rectangular prism of cheese taken along the length of the cheese and at or near the axis. This prism was divided into 1 in. and ½ in. portions which were ground in a mortar; and the salt and moisture contents were determined.

Table II. *Moisture and salt contents of different portions of a Cheddar cheese after immersion in brine.*

Position of sample		Portion 1	Portion 2	Portion 3	Portion 4
Top	Moisture	40·52, 40·05 } 40·3	39·7	39·5	—
	Salt	0·97, 0·96 } 0·97	0·53	0·11	0·10
	Salt/moisture (%)	2·41	1·33	0·28	—
Bottom	Moisture	39·75	39·3	39·2	39·2
	Salt	0·75	0·16	0·15	0·13
	Salt/moisture (%)	1·81	0·40	0·38	0·33
Side	Moisture	40·3	39·0	39·2	—
	Salt	1·02	0·15	0·15	0·15
	Salt/moisture (%)	2·53	0·38	0·38	—
Side	Moisture	40·05, 40·13 } 40·1	39·4	39·4	—
	Salt	0·99, 1·04 } 1·02	0·19	0·10	0·18
	Salt/moisture (%)	2·54	0·48	0·25	—

Wherever possible, estimations were carried out in duplicate. It was found that, with the first two cheeses, there were slits and fissures which tended to upset the results to some extent. Hence the last two series of analyses were made on transverse slices of the cheese from which about ¾ in. of rind had been removed.

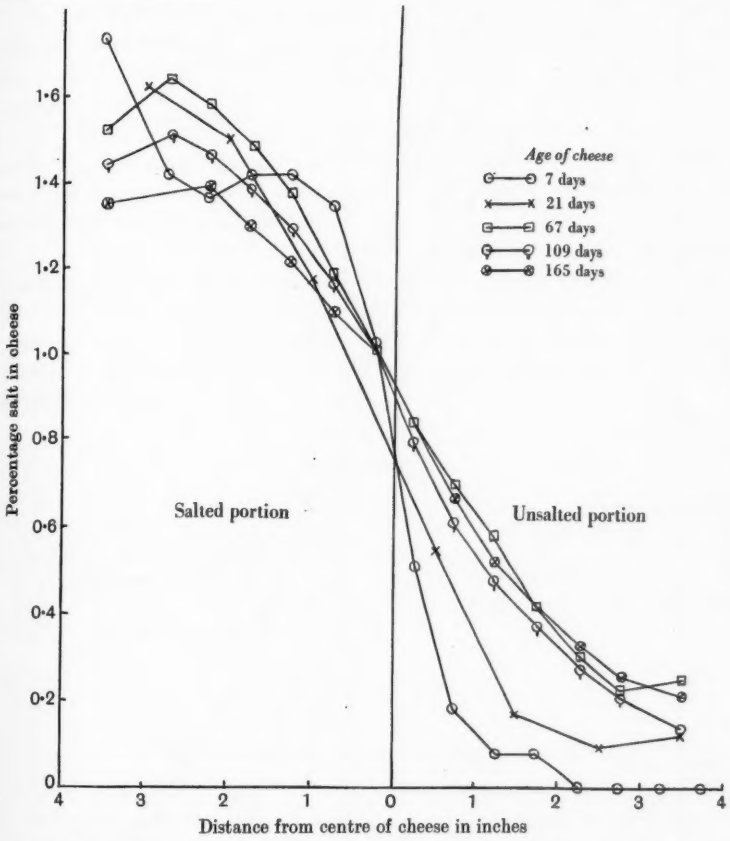
The results are presented in Graphs 2 and 3.

There is evident a gradual fall in the salt content of the salted portion and a gradual rise in the salt content of the unsalted portion. In 7 days the salt had not diffused further than 2 in. into the unsalted portion. In 21 days the salt content of the outer section of the unsalted portion had reached 0·12 per cent. and in 165 days this had increased to only 0·23 per cent.

The curves differ slightly from what might be expected, in that the fall in salt content of the salted portion is smaller than the rise in salt content of the unsalted portion; also, the curve for the unsalted portion at 67 days

lies higher than the curves for 109 days and 165 days. This may be due to one of several causes:

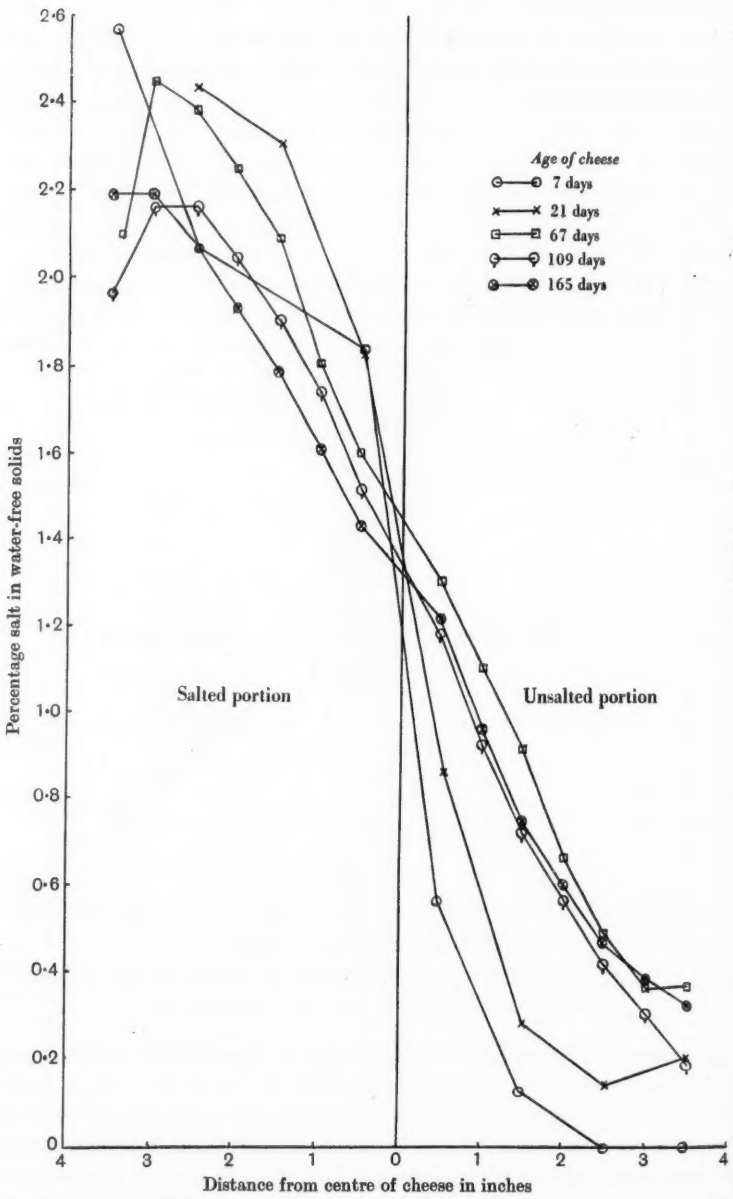
(*a*) It has been demonstrated that the salt content of a cheese or of cheeses from one vat show considerable variation, actually larger than the variations recorded here.



Graph 2. Graph showing the content of salt in salted and unsalted portions of a cheese at different positions in the cheese and at different ages.

(*b*) A small variation in salt content is to be expected due to the change in moisture content with shrinkage.

(*c*) The curves are not exactly comparable, since the sample was not taken in exactly the same position for the five analyses. It is to be expected that the greatest amount of diffusion will take place at the axis of the cheese. The first three analyses were carried out on relatively small prisms of cheese taken near the axis, whereas the last two series of analyses were made on a large block of cheese.



Graph 3. Graph showing the content of water-free solids in salted and unsalted portions of a cheese at different positions and at different ages.

Summary and conclusions.

1. Figures are presented which show that there is a very appreciable variation in the salt and moisture contents of small plug samples taken from different cheeses from the one vat.

2. The fact that a cheese plug has to be ground to a paste before good duplicate results for salt content can be obtained is a further illustration of the variation in salt content within a single cheese, and of the slowness of diffusion of salt in Cheddar cheese.

3. The extent of diffusion of salt into unsalted Cheddar cheese has been measured in two different experiments. The results show that diffusion is very much slower than in Limburger cheese, probably owing to differences in moisture content and texture.

4. It is well known that salt has an inhibiting action on the growth of bacteria in cheese. With variation in salt content from place to place in a cheese it is therefore to be expected that local variation will be met in the bacterial content. This may explain in part at any rate, the difficulties encountered in obtaining concordant results for estimations of number of bacteria in cheese.

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(*MS. received for publication August* 10, 1932.)

BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. THE PHYSIOLOGY OF DAIRY CATTLE.

I. Growth (by G. L. Peskett).

II. Lactation (by J. D. Stirling).

III. Nutrition:

(*a*) Digestion (by S. Morris).

(*b*) Feeding Standards (by J. Mackintosh).

IV. Genetics (by A. D. Buchanan Smith and Olive J. Robison).

636.2:612.65

I. GROWTH.

The work of the last three years on the growth of cattle can be reviewed from two aspects: (1) the general data on growth that have been collected, and (2) the observations that have been made on the influence of various factors, *e.g.* nutrition, on growth.

Growth data.

Turner and Herman (1), in studying the blood volume as a possible index of the productive ability of cattle, record that in young growing cattle the blood volume is 6·25 per cent. of the body weight, compared with 8·11 and 6·38 per cent. the average values obtained for lactating and non-lactating cows respectively. In all three classes the plasma volume was 60–65 per cent. of the whole blood.

Kislovsky and Larchin (2) have made a study of embryonic growth in cattle, and find that, in Domshino (a subdivision of Jarosslav) cattle, the growth curve from 40 to 270 days after conception shows three abrupt breaks, *i.e.* four growth periods or cycles. Comparing their data with those of Hammond (3) for Shorthorns and of Bergmann (4) for "Spotted Niederungsbreed" they found a general similarity, though variations were noted in the rate and duration of the individual growth periods.

In an attempt to provide a flexible growth standard of more general application than that of Eckles (5), Ragsdale and Regan (6) have collected data of the normal values of certain body dimensions (and in some cases body weights) in American Holstein and Jersey cattle at different ages. Their figures were collected from a number of Missouri farms and should form a useful standard of reference, as the number of animals studied was sufficiently large to show the extent of normal variations to be expected in such measurements. Brody and Ragsdale (7), in observations on Jersey, Holstein, Guernsey and Ayrshire cattle, have shown in addition that the body weight can be determined from various linear measurements, *e.g.* heart girth, height at withers, shoulder to ischium. On charts which show the relationship of these measurements for all four breeds, a single line can be drawn from which the body weight of other breeds can be predicted from a given linear measurement.

Additional data of weights of growing Jersey, Holstein and Guernsey cattle are recorded by Ragsdale (8). Similar measurements on English Shorthorns from birth

to 5 years of age have been reported by Bartlett and Jameson (9). Their results show close similarity to those of Eckles (5) for American Holsteins. Up to the 21st month the general trend of the curve of increase in live weight with age was a straight line, although during the first 6 months the average live weight increase per day tended to increase progressively. During this latter period there is, therefore, some advantage in expressing growth at a relative or percentage rate. This method was adopted by the authors for two groups of calves, one of which was fed heavily, milk feeding being continued for almost 6 months. The other group received average diet, the milk ration ceasing at about 10 weeks old. The relative growth rate remained constant throughout in the first group, whereas a definite abrupt change in growth rate occurred in the second group at the cessation of milk feeding, the relative growth rate continuing constant, but at a lower level, thereafter.

Influence of various factors on growth.

It has been observed (10) in America that heifers not exposed to direct sunlight have made slightly faster gains in weight than those which were exposed, possibly on account of freedom from flies and the cooler conditions.

An interesting hypothesis of the importance of water in growth has been put forward by Wohlbier (11), who points out that young animals contain more water than adults, the greater proportion of the water being bound to proteins in the body. When this proportion of water is very large growth can take place, but when, as in adults, it reaches a value of about 77 per cent. growth ceases. Wohlbier's theory is borne out by the observed fact that early gains in weight of young fattening stock consist of a greater proportion of water than later gains. (Wood (12).)

The food requirements of growing cattle are discussed elsewhere, though the following points may be added here. In making a survey in different countries Ashton (13) concluded that differences in size between cattle of different breeds may be due to differences in the supply of lime and phosphorus, characteristic of the soil of their country of origin. He found that in Brittany, for example, where the soil is poor in lime and phosphorus, the growth period was shortened compared with other countries, and the cattle and people were smaller. The work of Bartlett and Jameson (quoted above) suggests that the change from one cycle to another during growth may be due, at least in part, to changes in feeding, so that, at first sight, it is not unreasonable to assume that other fundamental problems of growth can be explained on a basis of feeding. At the same time Ashton's suggestion cannot explain the known inability of cattle such as Jerseys to grow above a certain (relatively small) size when they are receiving fully adequate rations.

Many observations on growth in relation to feeding have been conducted in the laboratory, using rats or other small animals, though it should be noted that these cannot be directly applied to cattle. A few may be mentioned here which appear to be of some agricultural interest. As regards the proportion of protein in the diet, Slonaker (14) reported that optimum growth of rats was obtained when this foodstuff constituted slightly more than 14 per cent. of the ration, and that progressive retardation of growth occurred if this proportion was varied by more than $\pm$ 4 per cent. Mitchell and Smuts (15), using rats, showed that soy bean, wheat, oats and corn are all deficient in amino acids essential to growth, as shown from experiments in which they were used individually as the sole source of protein in the diet. Belle (16) has reviewed statistics of growth of various animals, including cattle and man, in relation to the composition of the milk of the species. He concludes that the intensity of growth, *i.e.* the rapidity with which an animal doubles its weight at birth, varies with the richness of the mother's milk in protein and salts.

Two observations relating to the growth of milk-fed calves are of interest. Swesnikowa (17) fed one cow on a diet rich in vitamins and another on a vitamin-poor ration. Their milk was fed to calves and to rats. No difference could be detected in the growth, digestibility coefficient or metabolism of the calves receiving these milks, whereas the rats which were fed on the milk from the cow receiving the vitamin-rich diet grew quicker. Unfortunately the abstract of the paper, which is alone available to the present author, gives no details of which vitamins are concerned. American workers (18) claim to have shown that milk alone does not supply all the factors necessary for normal prolonged growth of calves, mineral supplements being considered necessary to raise a calf to more than a few months of age and to prevent the onset of anaemia. Such a claim is difficult to substantiate in view of the common practice of raising beef calves on whole milk for considerable periods.

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636.2:616.664.1

II. LACTATION.

THE PHYSIOLOGY OF MILK SECRETION.

(*a*) *Biophysical aspects.*

The course of milk secretion between two acts of milking has given rise to a surprising number of mutually exclusive theories, not one of which has been supported by decisive evidence. Thus it has been assumed that after the act of milking very active secretion continues until the udder is again filled, when secretion stops until the udder is emptied. Some writers hold that this filling of the milk reservoirs after milking is a rapid process, others that it takes place comparatively slowly. Controversy has also centred round the question as to whether secretion can take place during milking, but it has generally been assumed that secretion intensity is maximal at this time. The main argument of the upholders of the "two-phase" theory of milk secretion has been based on the oft-repeated statement that the

storage volume of the udder is very small compared with the volume of milk obtainable at a milking. Recent experiments carried out by Petersen, Palmer and Eckles (1) disprove this entirely. These authors made comparisons between the amounts of milk drawn (*a*) from each quarter for several days before slaughter, (*b*) from two quarters immediately prior to slaughter, and (*c*) from the two remaining quarters after removal of the complete udder from the slaughtered animal. The latter figure was obtained by excising the gland after slaughter, attaching it to a suitable frame, and hand-milking the two unmilked quarters. Under these conditions, the post-mortem milk yield from the two unmilked quarters was found to be at least as high as that from the two quarters milked immediately before slaughter, and this again approximated the yield obtained at normal milkings during the days preceding slaughter. Confirmatory experiments of the same nature carried out at the U.S. Bureau of Dairy Industry (2), where the total post-mortem milk yields from the excised udders were found to represent, on an average, 75 per cent. of the quantity given at ante-mortem milkings. The results of these studies indicate that milk secretion is continuous between milkings, and that a very large proportion of the milk obtained at any milking is present in the udder before the milking process is commenced. An interesting point brought out in these investigations is that the milk from amputated udders is normal except for very low fat contents. Extraction of such udder tissue by the usual fat solvents failed to reveal the presence of further amounts of fat and it would seem as if the rate of secretion of fat is greatest during the act of milking. The high percentage of fat in "strippings" cannot therefore be entirely due to mechanical separation of fat in the milk ducts between milkings.

Further evidence for the continuous nature of milk secretion is to be found in the work of Filipovic (3), who has applied the methods of mathematical analysis to the results obtained from two methods of milking. In the first series of observations each animal of a herd of fifteen cows was milked every half hour for 8 hours, all in a single day. In the second series each cow was milked at 11 a.m. on the first day of the experiment, at 11 a.m. and 12 noon on the second day, at 11 a.m. and 1 p.m. on the third day and so on for 8 days, the second milking each day taking place an hour later than on the day previous. Statistical treatment of the results show that the first method is quite valueless, for the disturbances caused by half-hour milking give rise to entirely unpredictable yields. The yields by the second method display a much more orderly progression, and the analysis of the arithmetical series so obtained throw much light on the course of milk secretion between two milkings. This is of a decided phasic nature, the greatest secretion intensity being reached 5 hours after milking when the secretion intensity has risen to fourteen times its initial value. It would be interesting to have the methods employed by Filipovic developed further in the study of milk yields. The finding that very little milk is actually secreted during milking is supported by the observations of Krzywanek and Brüggemann (4, 5). By taking manometric measurements these workers were able to follow the gradient of internal pressure of the udder contents during milkings. It was expected that any sudden rush of secretion during milking would be reflected in a sudden rise of pressure or at least in the maintenance of a constant pressure level over a considerable period. Instead it was found that the pressure within the milk ducts fell steadily during milking, and further that the pressure in any one quarter is independent of the other quarters.

At the present time very little is known of the physico-chemical mechanisms controlling milk secretion. A recent paper by Blackwood and Stirling (6) makes an attempt to discuss the secretion of milk from the point of view of physico-chemical theory. The nature of the transudate from the blood stream to the secreting cells of the mammary gland is considered, and it is concluded that the variation in

composition between milk and blood can best be explained by the assumption of preferential absorption of certain of the milk precursors. It is pointed out that the synthetic activities of the mammary gland involve osmotic pressure reducing reactions, and a theory of milk secretion is developed to connect these synthetic reactions with the known isotonicity of blood and milk. A brief treatment of the thermodynamic aspects of milk secretion is also included.

(*b*) *Biochemical aspects.*

It is generally agreed that the solution of many practical problems in milk production lies in a closer correlation of the yield and composition of the milk with the composition of the blood from which it has been derived. Such a correlation would entail more exact knowledge of the chemical nature of the precursors of milk constituents, and of the chemical and stereochemical transformations undergone by these precursors in the secreting cells of the mammary gland.

Recent work has drawn attention to the unsatisfactory state of our knowledge of the nature of milk precursors. The ideal method of determining which blood constituents are utilised by the active mammary gland is to analyse blood before entering and after leaving the gland. Owing to the inaccessibility of the afferent blood supply of the gland all previous workers have taken the blood of the jugular vein to represent mammary arterial blood, an assumption unsupported by any evidence. In a series of papers Blackwood and Stirling (7, 8, 9, 10) have shown that the metabolism of the tissues of the head and neck and the absorption of water by the salivary glands have a very decided modifying influence on the blood passing into the jugular vein. Jugular blood, therefore, cannot be taken to represent arterial blood. By comparing samples of blood taken simultaneously from the radial artery and the mammary vein of lactating and non-lactating animals it has been found possible to demonstrate the true extent of the absorption of blood solutes by the active mammary gland. On the average about one-seventh of the sugar and amino-acid nitrogen is absorbed during the passage of the blood through the gland of the lactating cow, the former being presumably utilised for synthesis of lactose and for providing energy, the latter for the synthesis of protein. In addition it is claimed that acid-soluble phosphorus compounds are utilised, and it is suggested that such compounds may be concerned in calcium secretion on the lines of Robinson's well-known theory of bone formation. In this connection it has previously been shown by Kay (11) that mammary tissue contains a phosphatase.

With regard to the enzymic reactions responsible for the synthesis of milk constituents little progress has as yet been made. The interesting stereochemical transformations which must be involved in the synthesis of lactose from blood sugar have received attention from two workers. Svanberg (12) has investigated the action of glandular extracts on glucose and galactose and on mixtures of these two sugars. In no case could any synthesis of lactose be noted. Winter (13), in the course of a study of the general metabolism of lactose, gives definite evidence for the appearance of this sugar after parturition and suggests that this may be occasioned by stoppage of some of the milk ducts during secretion and consequent resorption of milk constituents by the blood. This work raises again the question of why the mammary gland should go to the trouble of elaborating a specific carbohydrate, for it is shown that lactose administered *per os* is completely utilised, while it is excreted unchanged after intravenous injection. In a later paper (14) Winter records experiments with cellobiose. There are two possible methods whereby lactose might be synthesised from glucose: (1) galactose may first be formed and then condensed with glucose to give a β-galactoside, (2) no free galactose may be formed, but instead two molecules of glucose may first unite to give a β-glucoside, after which the H and OH

groups on the C_4 of the non-reducing moiety would be transformed to give lactose. If the latter be the normal course of events it is to be expected that the mammary gland would have the power of transforming cellobiose, which is a glucose β-glucoside, into lactose. The negative results obtained by Winter on incubating cellobiose with various extracts of mammary tissue would appear to indicate that the gland contains no agent capable of effecting stereochemical transformations in disaccharides.

The commercial and legal aspects of the fat content of milk has perhaps been primarily responsible for the considerable attention which has been paid during the period under review to the question of fat secretion. Maynard, Harrison and McCay (15) have carried out an investigation of the influence of the lactation cycle on the fatty acids, phosphatide fatty acids and cholesterol of the blood. They find that following parturition there is a rapid and approximately parallel rise in all these constituents, succeeded by a gradual drop to normal levels as the dry period is reached. Lactation has an effect on blood lipoids independent of any effect which changes in the fat intake may exert. Schaible (16) has recently studied the effect of lactation on the blood fat constituents. He finds that during lactation there is an increase in the amounts of total fatty acids, lipoid and neutral fats carried in the blood fat. Despite marked changes in the level of these constituents from the dry to the lactating state there is no change in the distribution of the fatty acids or in their nature as determined by the iodine number. The requirements for lactation so far as blood fat is concerned seem therefore to be increase in quantity without change in quality. McCay and Maynard (17) claim that the phospho-lipoid and total phosphorus contents of blood plasma depend on the intake of dietary fat, although no phosphorus compounds other than phosphatides are effected by changes in fat ingestion. Maynard and McCay (18) found that a ration low in fat lowered the milk yield but did not affect the fat percentage. The low milk yields were accompanied by low values for blood fat and blood cholesterol. Leroy and Marcq (19) claim to have established a high correlation between the percentage of fat in the milk and in the blood. Their work constitutes an attempt to explore the attractive suggestion that analysis of the blood of cow and bull calves would establish the value of individual animals for future milk production. It is doubtful if their evidence is sufficient for the practical application of such a test, and it should be noted that Porcher and Maynard (20) find no correlation between the fat content of the milk and the level of the blood lipoids at the time the milk is being secreted.

Gowen and Tobey (21) and Petersen, Palmer and Eckles (22) provide chemical evidence for the continuous nature of milk secretion. The former find that at the time of milking the udder contains the lactose equivalent of all the milk obtained at a milking. The composition of the dry udder is quite different from that of the active udder. Lactose is absent from the former, while the Reichert-Meissl number of the fat from the dry organ is quite different from that of the active tissue. The latter workers find that the fat of the lactating gland is intermediate in composition between butterfat and body fat, while the fat of the non-lactating gland is very similar to that of the surrounding adipose tissue. Two ambitious papers by Gowen and Tobey (23, 24) have appeared in the period under review. The work has been based on the thesis that any alteration in blood composition must necessarily be reflected in the composition of the milk secreted. Accordingly procedures likely to cause drastic alteration in the composition of the blood have been employed. These are (1) complete inanition, (2) administration of phloridzin, (3) administration of insulin. The results of these investigations are interesting in so far as they illustrate the effect of various drastic treatments of the animal organism on the secretion of milk, but it is extremely difficult to see how they contribute to our knowledge of the mechanisms which control the activity of the mammary gland.

(c) *The influence of hormones.*

It has been suggested for a considerable time that the initiation and cessation of milk secretion throughout the lactation cycle are regulated by hormones, but investigations in this field have been badly handicapped by the absence of criteria of purity of the hormones-containing tissue extracts employed. During the past two years there has been much activity in the field of the chemistry of the sex hormones, and the announcement of the preparation of highly purified and crystalline specimens of the oestrus-producing factor (25, 26) leads to the hope that it will soon be possible to investigate the effect of substances of known chemical constitution on the activity of the mammary gland.

Meanwhile further investigations have been carried out using organ extracts, but it can hardly be said that much progress has been made in unravelling the complicated subject of the hormonal control of milk secretion. It has emerged very clearly, however, that the state of activity of the mammary gland is conditioned directly by factors elaborated by the reproductive organs. Among these it would seem at the present time that the most important in their action on the mammary gland are the corpus luteum and placenta. From alcohol-ether-acetone extracts of these organs hormone preparations known variously as Menformon, Feminin, Placentin, Fontanin, and Oestranin, have been obtained. The effects of such preparations on the secretion of milk has been investigated by de Jongh and Laquer (27), Stückenberg (28) and Fellner (29). The last-named worker concludes that two factors are sent into the blood stream by the organs of reproduction, one stimulating the growth and development of the mammary gland during gestation, and the other inhibiting the secretion of milk by the developing gland. The removal of the placenta at parturition is supposed to terminate the secretion of the inhibiting factor into the blood stream, with the result that normal milk formation takes place. The investigations of Turner and his co-workers (30, 31, 32, 33, 34) fully bear out the existence of a growth-promoting substance elaborated in the corpus luteum, but the initiation of secretion is ascribed to the appearance of a stimulating agent rather than to the disappearance of an inhibiting one. The work of Grüeter and Stricker (35), Turner and Gardner (36) and Corner (37) identifies this active principle as a product of the anterior lobe of the pituitary. It would appear that two reproductive hormones are produced by this organ, one a gonad-stimulating hormone and the other having a direct action on the mammary gland. The latter is able to produce a flow of milk from the non-active gland artificially developed by means of placentin. It has to be stated that there still exists much confusion in this branch of the study of milk secretion and one cannot help but feel that the lack of standardisation has contributed to the contradictory results obtained by the use of different preparations by different workers. If the artificial control of milk secretion is to be achieved, and this would appear to be the reason for so much enthusiasm for research on the regulating hormones of the mammary gland, much more attention will have to be paid to the purity and reproducibility of the reagents employed.

The clinical chemistry of dairy cattle.

The past two years have been noteworthy for the number of attempts to apply the methods of blood analysis, of such great service in human physiology, to problems in the pathology and nutrition of dairy cattle and in the physiology of lactation. The consideration of the work in the latter field has already been dealt with, but it was felt that this section of the Reviews would be incomplete if it failed to survey the more general application of clinical chemistry to the physiology of dairy cattle.

It has been recognised that the first necessity for the extension of the use of clinical methods to dairy cattle is the establishing of normal values for the various

blood constituents. Thus Anderson, Gayley and Pratt (38) have analysed samples of blood from 59 animals and present normal values for haemoglobin, non-protein nitrogen, urea nitrogen, uric acid, creatine, creatinine, sugar, chlorides, phosphorus, calcium and carbon dioxide combining capacity. Allardyce, Fleming, Fowler and Clark (39) offer data of a similar nature. They record analyses for cholesterol, sugar, non-protein nitrogen, urea nitrogen, amino-acid nitrogen, creatine, creatinine, calcium, inorganic phosphorus and chlorides, while they note the effect of diet and of certain pathological conditions on some of these blood constituents. A very complete study of the blood sugar level of dairy cattle has been made by Hodgson, Riddell and Hughes (40) in which the influence of age, breed, ingestion of food, fasting, stage of lactation, oestrus, excitement and administration of glucose has been investigated. A study of the blood-sugar level during non-lactating and lactating periods, in oestrum and in certain pathological conditions such as pyelonephritis, sweet-clover poisoning and obscure paralysis has been made by Hewitt (41). Artificially induced hypoglycaemia and hypocalcaemia have been studied by Petersen, Hewitt, Boyd and Brown (42), and their work should dispel the lingering illusion that hypoglycaemia is associated in any way with milk fever. They have been able to show that the bovine tolerates a much lower blood-sugar level than is reported for other species. In prolonged cases of hypoglycaemia values for blood sugar as low as 17 mg. per 100 c.c. have been obtained, the symptoms being in no way analogous to those of milk fever. Further reports of blood studies in milk fever have recently been made by Wilson and Hart (43). Determinations of the haemoglobin content of the blood of dairy cattle have been made by Krauss and Hayden (44), McCay (45) and Brooks and Hughes (46). So far it has not been found possible to detect any significant variation in haemoglobin content. The contrast between haemoglobin and such metabolically active substances as calcium and phosphorus is apparent when we consider the work of Palmer, Eckles and their school. Palmer and Eckles (47) have shown that the calcium content of the blood of dairy cattle is subject to considerable variations, the cause of which cannot yet be stated. Palmer, Cunningham and Eckles (48) have studied the normal variations in inorganic phosphate content. Inorganic phosphorus varies not only from day to day but from hour to hour, and the latter workers have shown that exercise, feeding and parturition have very decided effects on this blood constituent. Palmer, Gortner and Rude (49) could find no correlation between the amounts of calcium and inorganic phosphorus in the blood plasma of dairy cattle. They state that no biologically significant amount of calcium phosphate occurs in the blood plasma of such animals, and they conclude that the mineralisation of bone is not explainable on the basis of a mere precipitation of bone salts from body fluids.

An important innovation in the field of dairy science is the application of the dye injection method to the study of the blood and plasma volumes of dairy cattle by Turner and Harman (50). The accumulation of data of this kind cannot but have importance in the future development of the study of the biological problem in milk production. An interesting example of the study of pathological states from the point of view of blood chemistry is the attempt which is at present being made to investigate the chemical basis of sweet-clover poisoning among dairy cattle. Papers on this subject have been published by Cannon and Greenwood (51) and Roderick (52). It has been established that sweet-clover poisoning is associated with prolonged coagulation time of the blood and low haemoglobin content, and the nature of the specific agent responsible for such interesting biochemical symptoms is at present being investigated.

As this short summary shows, the work in clinical chemistry at the present time is mainly concerned with the accumulation of data. It is to be hoped that it will

be found possible, in the near future, to apply the results of such observations to the solution of practical problems in the dairy industry.

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III. NUTRITION.

(*a*) The physiology of digestion.

The study of digestion in the ruminant is of comparatively recent origin. The importance of this subject cannot, however, be denied, since a knowledge of the metabolic processes which occur in the digestive tract must inevitably form the basis of future advances in the application of nutrition to the rationing of livestock.

Saliva.

In connection with salivary secretion, the work of Scheunert and his co-workers (1, 2, 3) is outstanding. Measurements of the amount of saliva, made by means of temporary parotid fistulae, showed that great variations occurred without any apparent reason, the amount of saliva secreted being independent of the animal's weight. During the course of the experiment the amount secreted decreased, and in 10 days the activity of the gland ceased. Rumination, however, could proceed by means of the other glands. Permanent fistulae resulted in death through loss of water and alkali. Contrary to non-ruminants, there was found to be no psychical secretion of saliva, and chemical stimuli had no effect on the salivary secretion of cattle, sheep and goats. Rumination only stimulated the side on which the animal chewed. Chemical analyses showed that the saliva was an almost pure solution of sodium and potassium bicarbonates and carbonates, with only small amounts of chlorine and phosphates. Brünnich and Winks (4), on the other hand, claim that the saliva of the sheep has a high phosphate content.

Nitrogen metabolism in the rumen.

In 1925 Schwarz (5) undertook an investigation into the protein distribution of rumen contents obtained after slaughter. He found that the average nitrogen content amounted to 0·13 per cent., which he classified as 9·1 per cent. unbound, 12 per cent. bacterial, 26·3 per cent. infusorial, and 58·6 per cent. fodder. Mangold and Schmitt-Krahmer (6) showed that the nitrogen content of the rumen varied with the feed but could not exceed a definite figure, 0·439 per cent. nitrogen with sheep. The authors considered this to be due to salivary dilution. Starvation for 4 days reduced the nitrogen to 0·05 per cent. Differentiation of the nitrogenous constituents gave 90–96 per cent. true protein, 4–10 per cent. amide nitrogen, and 0·043–0·052 per cent. ammonia nitrogen. The proportion of infusoria protein in the total amounted to 10–15 per cent. (*i.e.* with about 1000 infusoria per c.c.). In this connection it is of interest to note that Mangold and Usuelli (7) have recently shown that a milk diet causes death of the rumen infusoria, a result which they claim to be due to a decrease in *p*H. *In vitro* experiments gave a decrease in *p*H from 7·5–5·9 in 24–48 hours. Schwarz found an average of 11·7 per cent. for the ratio of bacterial to total nitrogen, a figure which agrees well with the later work of Mangold. It

appears therefore that approximately 30 per cent. of the nitrogen in the rumen is of micro-organic origin, and is easily broken down by the digestive juices in the abomasum and duodenum.

Cellulose fermentation.

The question of the part played by cellulose or fibre in animal nutrition is of extreme importance. It can no longer be denied that the function of the fibre is more than a mere filling of the rumen, and that it can undergo digestion and be transformed into nutrients useful to the animal. In most foods there is present a cytase capable of splitting up the cellulose (Brown and Morris (8)). This enzyme has also been shown to be present in the ruminant stomach (Karrer and Staub (9)), but there is no doubt that the main source of cellulose destruction is bacterial. The rumen and the intestines appear to be the only parts of the alimentary tract in which active digestion of cellulose occurs, a fact which is due to the *p*H, which approximates 7·5 in the rumen, 7·8 in the small intestine, and 8·4 in the large intestine. In the abomasum the *p*H is 3·8, and, since the limiting *p*H for bacterial activity is 4·8–5·0, no action occurs. Woodman and Stewart (10), however, found the organism alive in the abomasum. It would probably pass on to the intestines, where activity would once more commence. That the infusoria have no cellulose-splitting properties has been conclusively shown by Usuelli (11).

Considerable importance is attached to the course of destruction of fibre in the animal. The majority of *in vitro* investigations has been made on the organisms isolated from the faeces of ruminants, *i.e.* thermophilic bacteria which can break up cellulose at temperatures of 55–70° C. Viljoen *et al.* (12), for instance, isolated an organism which split cellulose to form acetic acid, CO_2 and hydrogen. A reaction of this type cannot, however, take place in the animal, according to Woodman (14), since the results would not tally with the classical feeding experiments of Kellner. Woodman has therefore proposed a new scheme to account for the equal production values of starch and cellulose, based on the following reactions:

$$\text{Cellulose} \rightarrow \text{cellobiose (glucose-}\beta\text{-glucoside)} \rightarrow \text{glucose.}$$

By careful control of the fermentation Woodman and Stewart (14) have shown that glucose is the final product, but that the digestion process is complex. The micro-organisms cannot form glucose themselves, but they contain a cyto-hydrolytic enzyme and probably a cellobiase, which cause the formation of glucose. In this connection the earlier work of Pringsheim (13) is suggestive. This author found that cessation of growth at the active stage, induced by volatile antiseptics, gave as intermediate products glucose and cellobiose.

Woodman and Stewart (10), in an attempt to measure the digestibility of fibre by *in vitro* methods, have studied the effect of thermophilic organisms on fibre separated from different foodstuffs, and on the original foods. With fibre alone, *in vitro* digestion at 65° C. for 14 days gave a higher fermentation coefficient than the corresponding fibre digestion coefficient in animal trials. This was probably the effect of the fibre being free from the other constituents. Using whole oil cake, *in vivo* digests gave a decreased digestibility of the fibre, a result which the authors believe to be due to the production of an insoluble substance from the oil, this substance being retained in the fibre residue after extraction.

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(*b*) Feeding standards.

Feeding standards for dairy cows.

In the first number of this *Journal* Halnan (1) contributed a detailed and critical survey of the literature dealing with feeding standards for dairy cows, and, as a result of his studies, suggested that the standard for maintenance commonly accepted in Great Britain, viz. 6 lb. starch equivalent and 0·6 lb. protein equivalent per 1000 lb. live weight, might be reduced to 5 lb. starch equivalent and 0·6 lb. protein equivalent, *i.e.* a reduction of the starch equivalent by one-sixth; in the production standards Halnan also suggested a slight reduction in the quantities of starch equivalent and protein equivalent per gallon of milk.

It is worthy of note that Halnan in fact suggests a greater reduction in the production standard per gallon of milk than at first sight appears to be the case, since he has assumed that 0·5 lb. digestible true protein is equal to 0·6 lb. protein equivalent. For foods of a bulky nature this relationship holds good, the protein equivalent being about 20 per cent. higher than the true protein. For concentrates, however, the difference between true protein and protein equivalent is much less, approximating 4 per cent. It follows that, in expressing the production requirements per gallon of milk (for which purpose concentrates are mainly used) the weight of protein equivalent should be only some 4 per cent. greater than the weight of pure protein. The effect of this correction, so far as Halnan's recommendations are concerned, would be to reduce his protein equivalent figures by about 13 per cent. Thus Halnan's production standard for milk of 3·5 per cent. butterfat would be reduced from 0·54 to 0·47 lb. protein equivalent per gallon. Rations made up on the revised scale would show a saving of some economic importance in the feeding of heavy yielding cows, but probably such a reduction under practical conditions should only be made where walking exercise is limited and the circumstances of management are highly favourable.

A further study of feeding standards for dairy cows has been made by Kriss (2) and by Forbes and Kriss (3), who have now formulated a revised basis for calculating food requirements. In their new standard, which is based on results of complete energy balances, the energy requirements for maintenance and for milk production

are both stated as metabolisable energy, the value for maintenance being 8·5 therms per 1000 lb. live weight, and for production 4·44 therms per 10 lb. of milk of 3·5 per cent. butterfat. In the computation of rations, the equivalent quantities of total digestible nutrients (the sum of the digestible carbohydrates, the digestible protein, and the digestible ether extract × 2·25) are used as a basis for calculation, the experimentally established factor relating metabolisable energy and total digestible nutrients being, for normal mixed rations, 1·616 therms per lb. On this basis the maintenance requirement works out at 5·26 lb. total digestible nutrients per 1000 lb. live weight, and the production requirement at 2·75 lb. total digestible nutrients per 10 lb. of milk of 3·5 per cent. butterfat. The protein requirement is given in terms of digestible crude protein, and the amount for milk production is based on the protein content of the milk × 1·25 for moderate production under optimum conditions, or × 1·75 for high rates of production and where the feeding stuffs are not of high quality and the food protein is of low biological value. For a milk of 3·5 per cent. butterfat, the protein requirement may therefore vary from 0·42 to 0·59 lb. digestible crude protein per 10 lb. milk. The suggestion that the standard allowance of digestible crude protein should vary in accordance with the level of production and the quality of the foods is a new feature, and worthy of careful consideration in the formulation of rations.

The problem of the protein supply needed for milk production has been studied experimentally in a different manner by Savage and Harrison (4). A herd of 36 pure bred and grade Holstein Friesian cows of mature age and capable of good milk production was divided into three uniform groups of 12 each. The same bulky foods were given to all groups at the rate of 1 lb. hay and 3 lb. silage for each 100 lb. live weight; the concentrate mixture consisted of the same foods, but the proportion of the ingredients was varied so that one group of cows received a mixture containing 16 per cent. total protein, another a mixture containing 20 per cent. and a third a mixture containing 24 per cent. The concentrates were given at the rate of 1 lb. for 3½ lb. milk produced. The protein fed to the respective groups was therefore equivalent to 0·45, 0·57 and 0·68 lb. total protein per 10 lb. milk. The cows were allowed some exercise but no pasture at any time.

This experiment has now been in progress two years and the results obtained in each year are given below:

	Group 16 % P lb.	Group 20 % P lb.	Group 24 % P lb.
First year: Average milk yield	9,261·5	10,055·8	9,399·4
Average fat yield	279·81	329·28	283·37
Second year: Average milk yield	9,558·1	9,690·8	9,803·7
Average fat yield	299·80	318·86	306·75

The differences in the above group averages are attributed to normal variations in groups of cows and are not considered significant; independent judges reported that at the end of the second year the conditions, general health and vigour of the cows were as good as at the beginning of the experiment. Savage also states that, when the protein for maintenance was deducted, the ratio of digestible protein consumed to the protein produced in the milk was as follows: 16 per cent. protein, 125 per cent.; 20 per cent. protein, 150 per cent.; and 24 per cent. protein, 188 per cent.

A report, with a summary in English, has recently been issued by Frederiksen (5), dealing with experiments on the feeding of cows in Denmark. The object of the experiment was to determine the requirements for milk production, in terms of feed units and protein, of cows kept under Danish farm conditions. The experiments were carried out during 1922–6 and involved almost 600 cows distributed amongst

a number of suitable farms. The group system was employed, and in the first series of experiments the amount of digestible true protein was varied in proportion to the yield of milk and the number of food units per kg. 4 per cent. milk was kept constant; in the second series the digestible true protein remained constant and the number of food units was varied in proportion to the milk yield, while in a third series the digestible true protein and the number of food units was raised in proportion to the yield. The conclusion drawn from the three series of experiments was that the requirement of feed for milk production of Danish cows maintained under farm conditions is 0·4 production food unit and 60 g. digestible pure protein (or 70 g. digestible crude protein) per kg. of 4 per cent. milk, which is equivalent to 2·78 lb. starch equivalent and 0·59 lb. digestible pure protein per 10 lb. milk.

Mineral requirements of dairy cows.

In a previous issue of this *Journal* Crichton (6) reviewed the present knowledge on this subject in a comprehensive monograph, and in a later issue (6a) a short review was given of two long-range experiments on the addition of mineral supplements—mainly steamed bone meal or flour and carbonate of lime—to the diet of dairy cows.

Recently another long-range mineral feeding experiment covering a period of 6 years has been reported by Hayden, Monroe and Crawford (7). The results are of special interest because the conditions of the experiment approximated closely to those obtaining on many milk-producing farms. A herd of Holstein Friesians, usually consisting of 12–18 cows, was divided into two groups approximately equal in milk production and both groups were fed alike except that the grain mixture given to one group contained 2 per cent. di-calcium phosphate. From May to October each year, when the herd was on pasture, the grain allowance was varied according to the condition of the pasture; hay was given when the pasture was short, and in the last two summers soya beans were given when necessary as a soiling crop. The di-calcium phosphate used was a special bone flour manufactured by precipitating the phosphate extracted from a very high grade of bone by the use of high grade chemical lime.

The data obtained from the milk production of the two groups did not show any increase in milk or fat that could be attributed to the di-calcium phosphate. With all the records computed to 4 per cent. fat-corrected milk at a mature age and covering the first full 7 months after calving, the average daily production was, for the mineral group, 37·3 lb. milk, and for the non-mineral group 39·8 lb. milk. The difference of 2·5 lb. favouring the non-mineral group was not considered significant. A comparison of 4 per cent. milk yield of 12 cows before and after feeding the mineral supplement as well as a comparison of the first and second lactations of the heifers introduced into the two groups, failed to show any superiority of the mineral ration. The health records of the two groups and the appearance and vitality of the calves born were on an equality, but there was a slight indication that the di-calcium phosphate had some beneficial effect on conception. The records show that the average number of matings per calf in the mineral group was 1·53 and in the non-mineral group 2·13. It is considered by the authors that this difference is slightly significant from the statistical standpoint.

It has become customary to include iodine when considering the mineral requirements of dairy cattle, and the interim results obtained in an experiment on supplementary iodine feeding were recently reported by Crichton (8). A lot of 20 Ayrshires—16 first calf heifers, two second calf cows and two third calf cows—was divided into two groups comparable as to age and as far as possible as to quality and probable milk production. Both groups were housed, managed and fed similarly

and no food known to be high in iodine was given; group I was given 0·1 g. KI per head per day while group II acted as controls.

The experiment has now been continued over two lactations and is still in progress. Although there is an indication that iodine had a beneficial effect on the health and percentage of butterfat of the milk of those cows under experimental conditions the author does not consider that our present knowledge warrants a general practice of feeding supplementary iodine to dairy cows. It is suggested that no conclusions can be formed until long time experiments in different areas and with different rations have been carried out.

Feeding standards for young dairy stock.

The maintenance requirements of dairy heifers has been studied by Eckles and Gullicksen (9). Results from 31 trials with animals of varying ages and weighing from 87 to 860 lb. are reported. The live weight method of determining the maintenance requirement was adopted in preference to balance trials because a larger number of animals could be used. The results indicate that when the revised net energy value of alfalfa hay (as determined by Forbes and Kriss) was used in calculating the nutrient value of the rations, the requirements for maintenance under the conditions of the experiment were slightly lower than the requirement of the Armsby standard at weights below 350 lb. and considerably above it at higher weights.

The same workers (10) have also studied the nutrient requirements for normal growth of young dairy heifers in a series of experiments extending over a period of almost 10 years and involving nearly 50 animals. Comparisons were made between the feeding standards for growing dairy heifers suggested from time to time by Wolff-Lehmann, Kellner, Armsby and Morrison and the results obtained showed that the nutrient requirements in terms of total digestible nutrients (digestible crude protein plus digestible carbohydrate and fibre plus digestible fat × 2·25) given in the Morrison standards are too low for the normal growth of dairy heifers up to about one year of age and too high beyond that age. The normal growth standard used was that ascertained by Eckles (10*a*).

The mineral requirements of young dairy stock.

The calcium and phosphorus requirements of dairy heifers during the first 3 years of life have been studied by Lindsay, Archibald and Nelson (11). Two groups of Holstein heifers were used; each group consisted of four animals and the experiment covered the period from the time they were weaned from milk (5–6 months) until the first calving (approximately 28 months). One group received a ration high in calcium and the other group a ration low in calcium. Metabolism balance trials were carried out at intervals during the experimental period for nitrogen, calcium, phosphorus and magnesium. With regard to calcium the average intake per 100 lb. live weight daily was 5·97 g. in the high calcium group and 3·17 g. in the low calcium group, and of this amount 17·8 per cent. and 21·6 per cent. were retained respectively. The phosphorus intake per unit of weight of the high calcium group was 21 per cent. less than that of the low calcium group, yet the former retained appreciably more of this element. In spite of the difference in the intake levels, the retention calcium/phosphorus ratio in both groups was slightly more than 2:1, and it is inferred that a lowered calcium intake will pull down the phosphorus retention, irrespective of the level of phosphorus given, to a point where the retention ratio is 2:1. It is also suggested that a high calcium content in the ration may be necessary in order to ensure adequate storage of phosphorus by growing heifers.

The results obtained also show that efficiency in the relative retention of the elements was lowered with advancing age.

All the heifers were weighed and measured at monthly intervals and were mated to calve when 28–29 months old. All gave birth to good calves and there was no significant difference in the live weights of the calves at birth. A study of the growth records and close observation of the animals themselves, indicate that, thus far at least, the considerably lower mineral storage by the low calcium group has had no ill effects. The rations given supplied approximately 4·5 g. calcium daily during the first year, 3 g. daily during the second year and 2·5 g. daily during the third year for each 100 lb. live weight.

The effects of rations containing different amounts of calcium and phosphorus on the growth of dairy heifers have also been studied by Henderson and Weakley (12). A lot of 32 grade Holstein calves were divided into four groups of eight animals each. Group I was given a normal ration which had been widely used with satisfactory results, while group II was given a ration much lower in calcium content. Group III was intended to receive a ration low in phosphorus but during the first year the washing to which the bran was subjected failed to lessen the phosphorus content sufficiently for the purposes of the experiment; the replacement of bran by dried beet pulp gave a ration low in phosphorus during the second year. Group IV was intended to receive a ration low in calcium and phosphorus but for the same reason as in group III, during the first year the ration was not deficient in phosphorus.

Records of live weight and height at withers, which were taken weekly, showed that groups I and III were somewhat above the Eckles standard towards the end of the second year, whereas groups II and IV failed to reach the Eckles normal in weight or height. Animals in group III, which were given a ration low in phosphorus (0·131 per cent.) during the second year, continued to grow almost as well as normal fed animals, even though the inorganic phosphorus content of their blood was much below normal. Rations containing as little as 0·25 per cent. of calcium did not affect the calcium content of the blood, though rations containing less than 0·35 per cent. of calcium or less than 0·20 per cent. of phosphorus produced a bone low in ash and consequently low in calcium and phosphorus.

Influence of certain foods on butterfat production.

The specific effects of coconut cake and palm kernel cake, given singly or as a mixture, on butterfat production has recently been studied by Schmidt and Vogel (13). When either of these foods or a mixture of both in quantities of 3 kg. per day were fed, all cows (with one exception) showed an increase in the fat content of the milk and in the quantity of milk fat; the degree of increase varied with different animals. It is also reported by Honcamp, Helms and Malkomesius (14) that the fat content of milk was not increased by the feeding of palm kernel cake containing 0·93 per cent. of fat, but samples having 7 and 16 per cent. of fat produced proportionate increases in milk yield and fat content. On the other hand, Perkins (15), reporting on an experiment with coconut meal in Ohio, failed to find any evidence that the percentage of fat in the milk was increased.

The conflicting results obtained in experiments designed to determine the effect of the above-mentioned foods on the fat content of milk, not only in the instances referred to above, but in numerous other instances, constitutes one of the puzzles of present day experimental work on milk production. Continental experiments have given results showing an increase in the fat content; English and American experiments fail to confirm this result. The explanation of this difference remains to be discovered, though as a result of recent work, Sheehy makes an interesting suggestion. Sheehy (16) noted the effect on the milk fat yield and percentage when

different fats were given in the ration in place of an equivalent quantity of maize or barley. In the great majority of cases the feeding of fat had no influence whatsoever. The effect of a daily dose of 6–8 oz. of cod-liver oil in depressing the fat content (17) was confirmed. Linseed oil, at the rate of 15 oz. daily produced a lesser but definite depressing effect after 10–12 days' feeding. In certain cases the feeding of oil appeared to increase the percentage of butterfat; some of these are accounted for by a simultaneous decrease in the milk yield and the remainder are attributed to normal variations in butterfat yield which accidentally synchronised with changes in feeding. Sheehy suggests that the synchronising of such normal variations with the start of an experimental period explains the apparently positive effects obtained in this and in similar investigations by other workers.

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IV. GENETICS.

TOTAL YIELD AND ITS RELATION TO BUTTERFAT YIELD.

The evidence is clear that total yield of milk is conditioned by genetic factors. In support of this, many experiments might be cited, but perhaps the most definite is that conducted at the Iowa Agricultural Experiment Station by Kildee, McCandlish and others. Hittcher (1) was the first to draw attention to the independent transmission of the characters of milk and butterfat, while the first sound evidence that butterfat is to a great extent inherited independently, appears to come from the Iowa experiments already cited. This belief has been confirmed by several subsequent investigators including Graves, at the United States Bureau of Dairy Industry, and Funkquist in Sweden. Up to the present time, no substantial evidence has been advanced to disprove this lack of relationship.

Number of factors involved.

Hansen (2, 3) accepts the theory that milk yield and butterfat content are each dependent upon triple homomeric factors. Each female must have the characters responsible for a minimum amount of milk, which he designated *M* or *m*, and the factor for fat is expressed as *F* or *f*. Three grades of each are possible:

$$(M_3M_3 \ldots m_3m_3; \text{ or } F_3F_3 \ldots f_3f_3).$$

Von Patow (4, 5) formulates an interesting theory, the validity of which he tests by applying it to some excellent data. He states that every cow must produce at least a minimum amount of milk, and that it is as impossible to have a cow without the ability to produce milk as it is to have milk entirely fat-free. He uses the letter *G* to designate the factor for the basic milk yield in cattle. This factor is always present in the homozygous state, otherwise some animals would be born which could not produce milk. Increase in milk yield is brought about by the presence of all, or some, of three homomeric and equally potent factors—*A*, *B* and *C*. These factors may be present in varying states of homozygosity, and doubt is expressed as to whether they are linked. Von Patow takes these three factors as corresponding to the three main characters which determine milk yield; viz. (1) development of the udder to allow for sufficient capacity of glandular tissue to secrete milk, (2) the formation of interstices, and (3) the innervation of the udder. The effect of these three factors is cumulative, and the presence of any one factor raises the milk yield above the basic quantity (4·6 kg.) by one unit (1·3 kg.) if in the simplex state, and by two units (2·6 kg.) if in the duplex state. Further, von Patow designates the factor for butterfat by the letter *F*, which may be present in three states, *FF*, *Ff*, or *ff*, each differing in the degree of intensity.

Turner (6) and Turner and Gifford (7), dealing with the mode of inheritance of butterfat, hold that milk and fat yield are influenced by many genes; many genes favouring high production are dominant, and all genes do not have the same effect. These authors assume the presence of three genes, *Aa*, *Bb* and *Cc*. Gene $a = b = c =$ 50 lb. of fat. In the dominant state, Gene $A = B = C = 250$ lb.

Constituents other than butterfat.

Very little work has been done along these lines, and most of it is the by-product of nutritional or environmental investigations. Crichton (8), in his review of the literature dealing with the mineral requirements of dairy cattle touches briefly upon this subject, and quotes the results of many workers who come to the conclusion that the ash content of milk cannot be greatly varied by nutrition. Since variations do occur, it is not unreasonable to attribute them in part to heredity. Evidence similar to this may be found in the paper by Allen (9).

Yapp and Kuhlmann (10) state that cows tend to give milk of much the same colour throughout their productive life, and that there is a tendency for related individuals to show a similarity in the colour of their milk.

Turner and Haskell (11) measured the diameter of the fat globule, and found a difference in the average diameter of the different breeds. This is confirmed by the work of Campbell and Yapp (12) and later Campbell (12*a*) who show that rate of decrease in the size of globules occurring with the advance in lactation is influenced both by breed and nutrition. According to Davis and Hathaway (13), breed differences in the vitamin content of milk appear to be insignificant.

Persistency.

For a long time it has been recognised by practical dairy breeders that certain cows have a tendency to become dry more quickly than others, and also that such cows have, during the period of highest daily yield, a higher yield than those cows which are more persistent in their daily yield. Sanders (25) has made a study of the "shape figure," and discriminates between cows which are "maximum" and "persistent" yielders. He alone seems to hold that persistency is due to a nutritional rather than to a genetic factor. Gaines (14) indicates that persistency is inherited through the female rather than through the male, and later, the same writer (15, 16), by correlating the yields of the first and second lactation, confirms his previous opinion. As reported by Yapp (17), the first generation cows of the Bowlker herd appear to have a greater degree of persistency than those of the second generation.

Becker and McGilliard (18) state that both the sire and the dam contribute to the inherited persistency of the offspring, and that maximum yield is also a genetic character. Turner (19) states that the rate of decline in milk secretion is due to further divisions of cells which become functional through the stimulus of the "follicular" hormone, and that there is a distinct decline in persistency during the second and third lactations as compared with the first. He considers that persistency and maximum yield are not linked in inheritance, and that with an increase in the dam's persistency, there is only a small increase in the persistency of the daughter. He also finds that overfeeding has no effect upon persistency, which shows marked variation amongst individuals subject to the same condition of feeding and management.

Conformation.

In practically all countries, except perhaps Denmark, type is still one of the most influential factors in selection. However, with the development of milk recording schemes, the value of performance records as an indication of the possible genotype of an animal is being gradually recognised. Larsen (20) has given a full account of the Danish methods of testing dairy cattle, and shows that the practice of judging sires purely on their appearance is a dangerous one so far as the improvement of milk yield is concerned. Prentice (21) has given an interesting history of "formalism" as applied to cattle. In his opinion the present score card is merely a late survival of the past belief in an association of form and function so close as to enable skilful persons to distinguish between good and bad animals. He argues that since the type desired for dairy cattle was almost identical with that desired for beef and draught, it was obviously ridiculous. Gowen (22) has studied this question in some detail, and has come to the conclusion that the reliability of body signs as indicators of probable milk yield is not great, even a short time milk record having far more reliability. He agrees with Turner in finding that the effect of the sire's conformation on his daughters is limited mainly to size. The heavier the sire, the greater the chance that his daughters will be high yielders both of milk and fat, but this indication has only about half the value of actual records. Recent work of Gowen (23) indicates that weight and wedge-shaped form, when viewed from the front, are indicative of milk yield. All correlations of other dimensions and milk yield are materially affected by age.

McDowell (24), using the records of over 100,000 cows, concludes that the larger cows usually excel the smaller ones in both milk and butterfat production. Gaines (16) has studied the question of efficiency of milk yield in which he combines the yield of butterfat with that of milk, and states that there is a distinct tendency towards

decreased efficiency with increase in live weight in all breed groups. Graves has shown that the shape of a heifer is liable to change. Thus a 12-month-old heifer might have sloping rumps, but this was no indication of whether or not the rumps would be sloped when she was 2 years of age. Yapp (17) is investigating the development of the udder with increase in age. For this purpose he takes, at regular intervals, paraffin wax casts of the udders of young heifers.

The work of Ivanova (26, 27) and Juler (28) are worthy of mention in that they deal with the inheritance of supernumerary teats but their results are contradictory. The former writer showed that the asymmetry of udder development is hereditary but obtained a small correlation between this and milk yield. Hammond (29) has given an instance of the transmission of supernumerary nipples by a bull to his daughters and also quotes other authorities. He concludes that the action of supernumerary nipples is one of lowering yield by preventing the maximum development of the normal glands. Richter (30) examined many males and females, and found no correlation between the presence of supernumerary teats and milk or fat yield.

The theory that the escutcheon was a sure indication of milk yield, was originated by Guenon at the beginning of the nineteenth century. Little work has been done recently upon this, but, where adequate scientific investigations have been carried out, such as those of Hooper at the Kentucky Station (31), Leroy (32), and Loesing (33), no evidence has been obtained to support it.

The relation of colour to production is a matter for some debate. Vogel (34), Lauprecht (35) and Prawochenski (36) deal with this matter. There is probably little connection, and certainly none has been proved to exist.

Duerst (37), who studied goniometer measurements in cattle, states that the characteristic angle of the rib is transmitted by the sire to his daughter, and by the dam to her son—the angle of the rib being actually the axis of the last rib with the horizontal. The relationship between dairy aptitude and rib angle has been confirmed by Déchambre (38), but Kronacher, Böttger and von Patow (39) were not able to find any relation between the angle of the last rib and the milk yield. Neumer (40) considers this measurement to be useless when applied to individuals or breeds, but useful as a general indication. Ante- and post-mortem experiments on dairy cattle have been carried out in South Carolina (41, 42) which indicate differences in the internal anatomy as measured by length of small intestine.

Mention must be made of the work of Swett, Graves and Miller (43) on the comparison of the internal structure of a highly specialised dairy cow with that of a highly specialised beef cow. They found the skeletal structure of the two animals to be much the same. Their extreme difference must be attributed in no small measure to the differing actions of the various organs of internal secretion. This links up with the recent advances in other fields of animal genetics which seem to show that in many cases the action of certain genes on the characters in question, is not a direct one. Certain genes act on the phenotype as a result of their action upon the organs of internal secretion.

Selahattin (44) concludes that good milking cows have larger thyroids and fewer non-medulated hair fibres, but he found no correlation between milk yield and the ratio of medulla thickness to hair thickness. Feuersänger (45) demonstrates that phenotype, as measured by skin thickness, milk veins, udder, etc., is of great significance in the estimation of the present or future milk production. Loesing (33) found no significant correlation between thickness of skin, rib angle, escutcheon, chest capacity, etc., and milk yield.

The progeny test.

Within the last few years there has been a great deal written about the value of the progeny test. All these investigations go to show the value of this test. Amongst them may be mentioned that of Atkeson, Mathieson and Fourt (47) who studied 3700 Idaho cows, Schöningh (48) (Black Pied East Friesian cattle data), Faber (49) (Danish methods), Meade (50) (Guernsey data), Davidson (51), Carroll (52) (Holstein-Friesian), Yapp (53), M'Candlish (54, 55), Hewitt (56) (Red Poll), Scott (57) (South Devon) and Edwards (57*a*). These are all unanimous about the value of the progeny test as a means of practical improvement.

At the present moment there is much dispute as to the best means of evaluating a sire by the production of his daughters, but it cannot be said that, as yet, any satisfactory method has been produced. The general feeling amongst those most qualified to speak on the subject appears to be that, while some form of definite index is desirable, such a thing is unattainable in practice. There are so many variables which must be considered, such as total yield, butterfat yield, whether the bulls were mated to high-yielding or low-yielding cows, etc., and the number of dam-daughter pairs necessary. There is no evidence to show that the sons of proven good sires are genotypically worse or better than the average of the breed.

Inbreeding and outbreeding.

It is remarkable how little inbreeding has taken place in the construction of the dairy breeds. On the whole, the balance of evidence is certainly against the practice of inbreeding. As a rule, inbreeding does not appear to be followed by an increase in production. Smith (58), as a result of an inbreeding analysis of the Jersey breed, has shown that high-producing cows are somewhat less inbred than are the average cows of the breed. In a recent paper, that of Hewitt (56), of the three methods of pure breeding (outcrossing, line breeding and close breeding), line breeding was found to give the most satisfactory results, as both milk yield and fat yield were increased.

Similarly, it must be noticed that outbreeding or cross breeding between breeds does not lead to hybrid vigour as expressed by increased milk yield. It is probable that these points are of fundamental importance.

Sex-linkage.

The lack of inbreeding and heterosis is one of the arguments used by Smith and Robison (59), who postulate the probable existence of sex-linked genes which, in part, control the yield of milk. If the organs of lactation are dependent for their expression upon a few genes, acting in either a definitely dominant or definitely recessive manner, there would be noticed in the first crosses of breeds a resemblance between the progeny and one of the parents. If yield and quality of milk were also dependent upon many factors expressing dominance or the absence of it, but not recessivity, then inbreeding would give good results. This is not the case, though there is not the slightest evidence to show that the proportion of recessive lethals is greater in the dairy than in the beef breeds. At first sight, the accepted fact of the existence of prepotent bulls implies that the factors governing the transmission of milk yield must behave in a dominant manner. But since these genes cannot be inherited in a dominant manner, the existence of prepotent sires can only be explained on the hypothesis that certain of the genes are transmitted in a sex-linked manner.

To test this, Smith and others have correlated the yields of granddaughters to those of their grandparents. Lower correlations have been obtained to the paternal

grandsire than to the other grandparents. Since the paternal grandsire cannot transmit a sex-chromosome to his granddaughter, these low correlations may be explained on a sex-linked hypothesis. Smith and Robison (59) employed a new method of comparison devised by Dr R. A. Fisher. This is based upon the differences in the yields of animals similarly related. Here again they obtain some evidence to show the effect of sex-linked factors as regards the inheritance of total yield. In respect of total yield of butterfat, the analysis of their data showed no evidence which involved other than autosomal genes. The conclusion at which these authors arrive is not very definite, since they refuse to accept the statistical evidence as conclusive. They point out, however, that it is reasonable to assume that a few of the factors affecting the inheritance of milk yield may be transmitted in a sex-linked manner, and that no evidence has been obtained which tends to disprove this hypothesis. The more recent work of Madsen (46) and the report of Taufer (60) tend to confirm these findings.

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The Editors have received the following:

(1) *Prüfungen an Hoch- und Momenterhitzern* (price 5 RM.) from the Prussian Dairy Research and Experimental Station, Kiel.

(2) Particulars of Photographic Series No. 8, showing various agricultural machines and buildings, also from the Prussian Dairy Research and Experimental Station, Kiel.